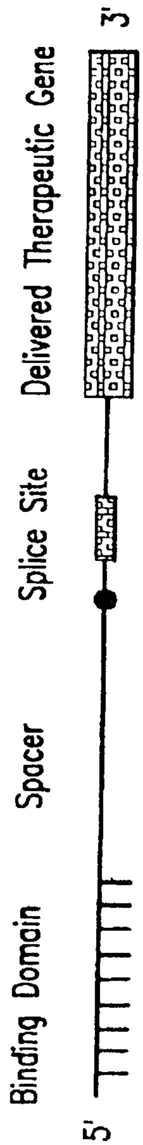

5'
Binding Domain
Spacer
Splice Site
Delivered Therapeutic Gene
3'

FIG.1A

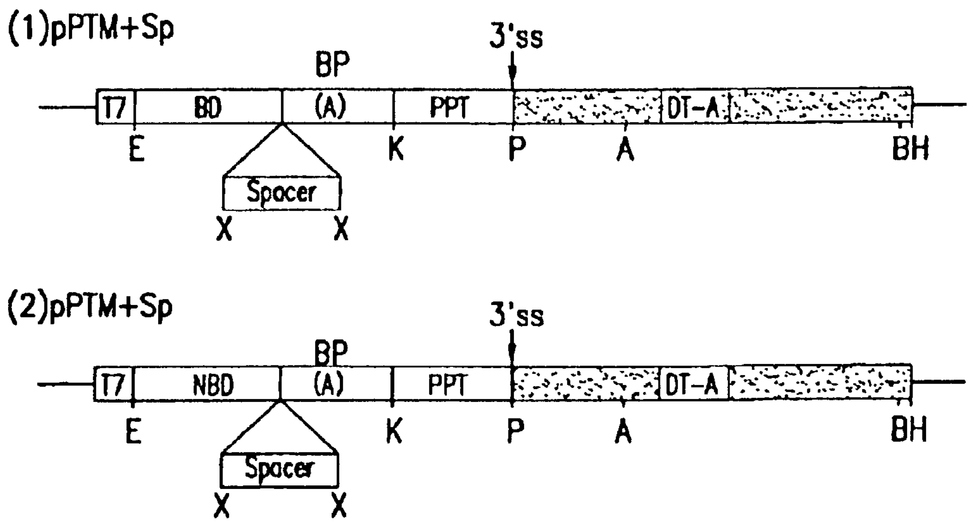

(1)pPTM+Sp
3'ss
BP
T7
BD
(A)
PPT
DT-A
E
K
P
A
BH
Spacer
X
X
(2)pPTM+Sp
3'ss
BP
T7
NBD
(A)
PPT
DT-A
E
K
P
A
BH
Spacer
X
X

FIG.1B

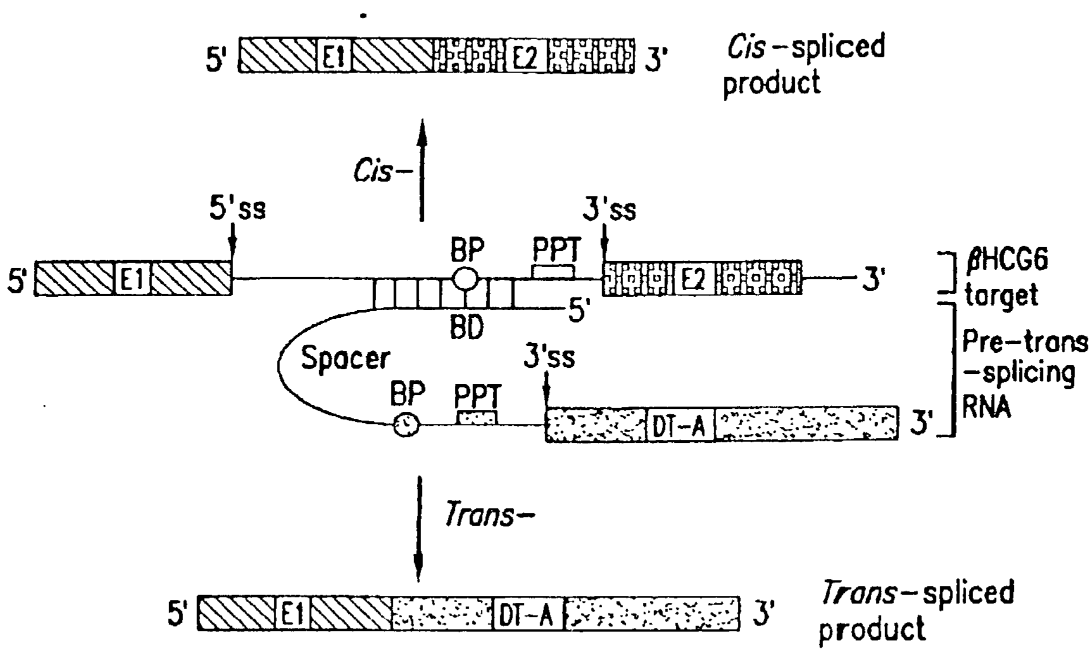

5'
E1
E2
3'
Cis–spliced product
Cis–
5'ss
3'ss
BP
PPT
5'
E1
E2
3'
βHCG6 target
5'
BD
Spacer
3'ss
BP
PPT
DT-A
3'
Pre–trans –splicing RNA
Trans–
5'
E1
DT-A
3'
Trans–spliced product

FIG.1C

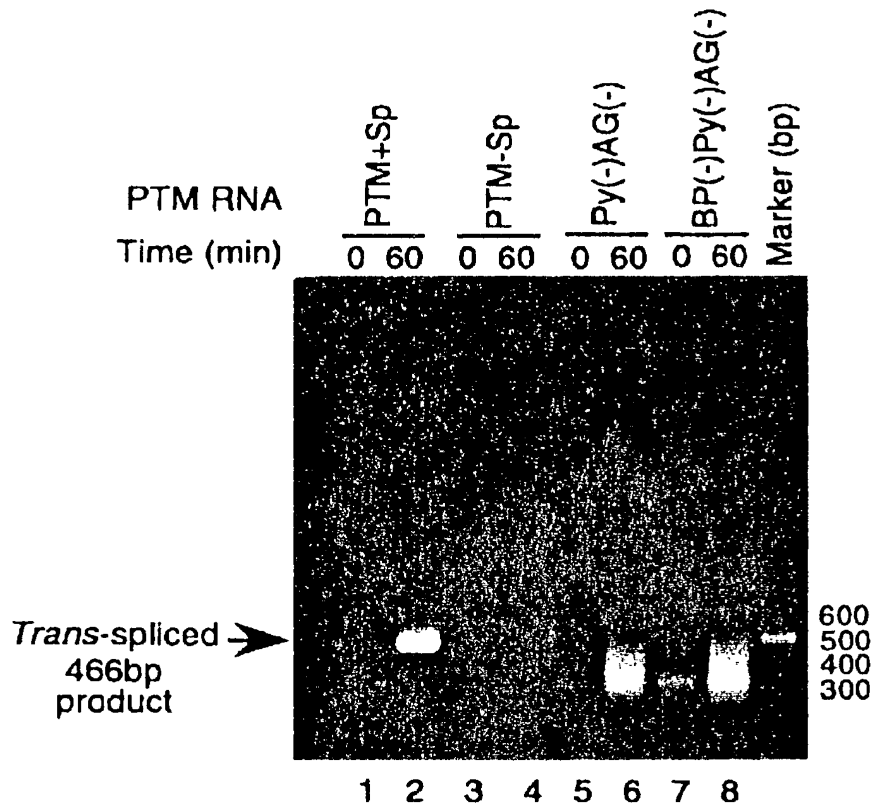

PTM RNA
PTM+Sp
PTM-Sp
Py(-)AG(-)
BP(-)Py(-)AG(-)
Marker (bp)
Time (min)
0 60
0 60
0 60
0 60
Trans-spliced 466bp product
600
500
400
300
1 2 3 4 5 6 7 8

FIG.2A

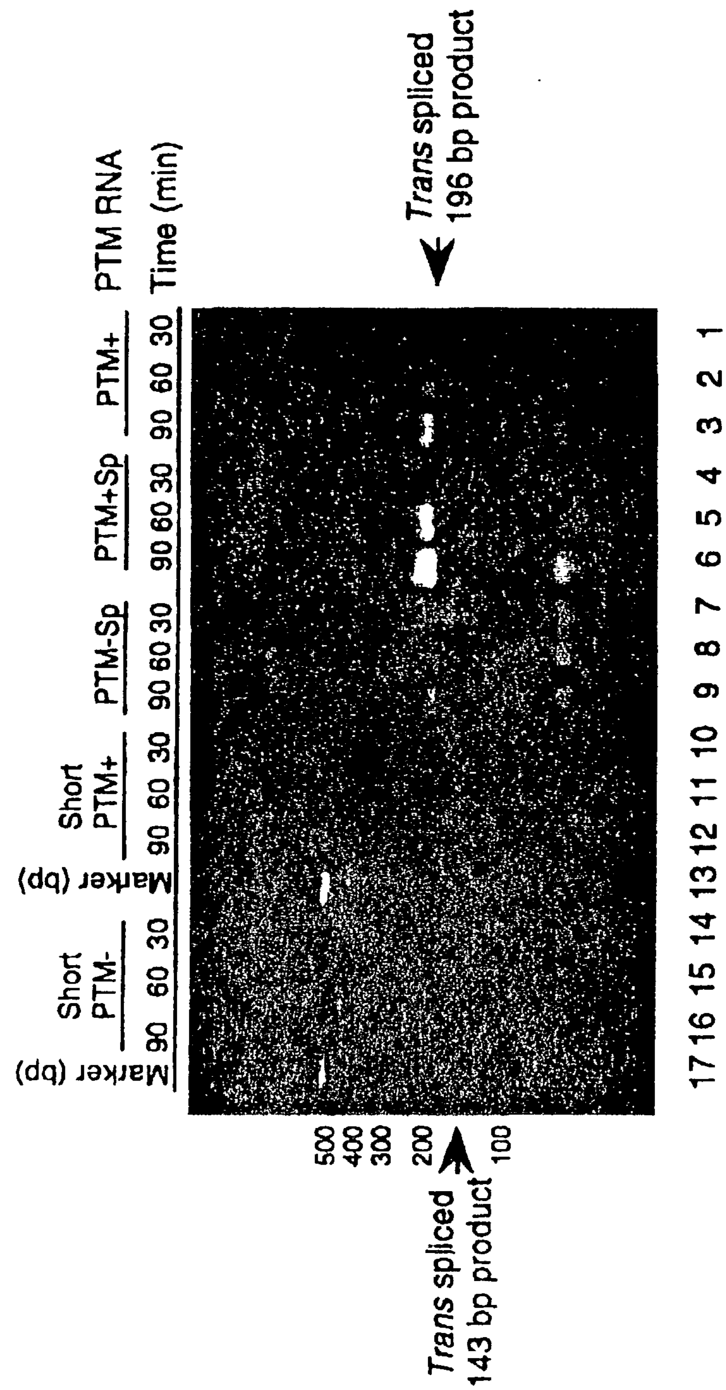

Marker (bp)
Short PTM-
90 60 30
Marker (bp)
Short PTM+
90 60 30
PTM-Sp
90 60 30
PTM+Sp
90 60 30
PTM+
90 60 30
PTM RNA
Time (min)
Trans spliced 196 bp product
500
400
300
200
100
Trans spliced 143 bp product
17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

FIG.2B

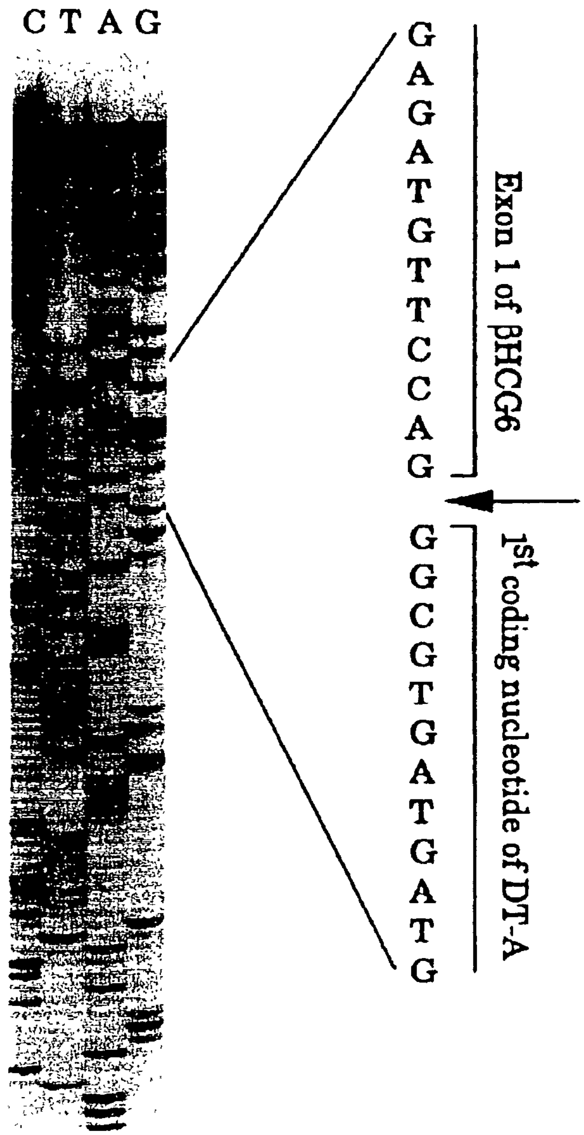

C T A G
G A G A T G T T C C A G
Exon 1 of βHCG6
G G C G T G A T G A T G
1st coding nucleotide of DT-A

FIG.3

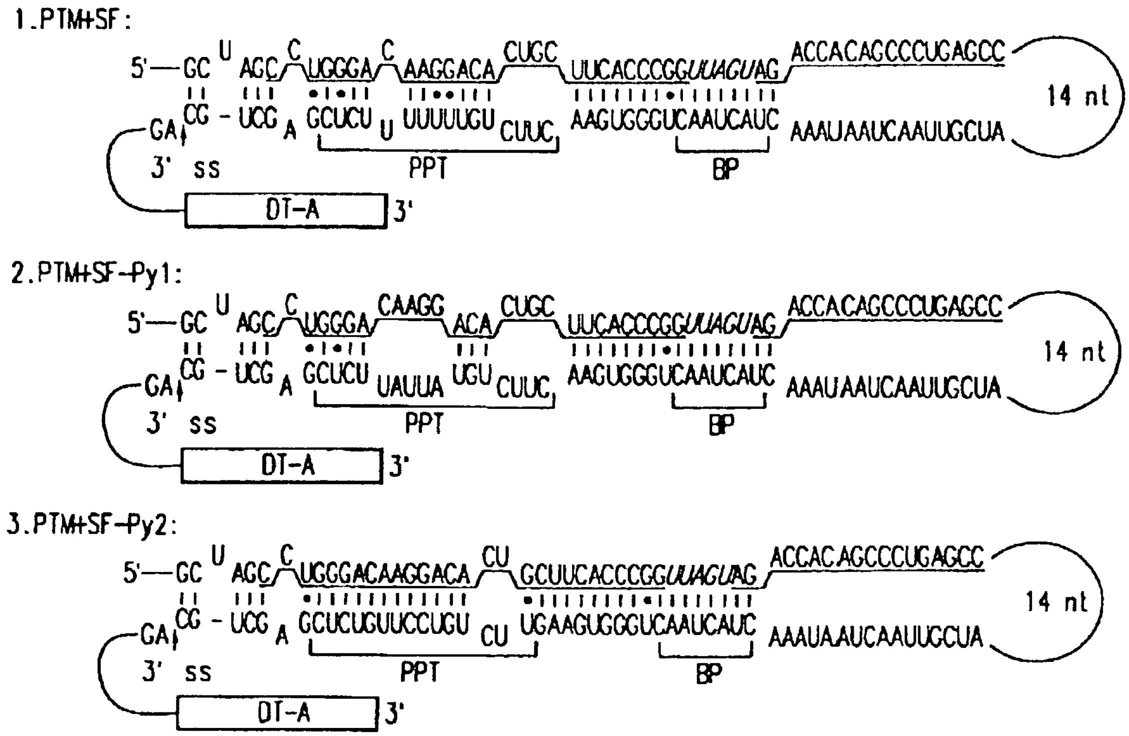

1.PTM+SF:
5'—GC U AGC C UGGGA C AAGGACA CUGC UUCACCCGGUUAGUAG ACCACAGCCCUGAGCC
GA CG - UCG A GCUCU U UUUUUGU CUUC AAGUGGGUCAAUCAUC AAAUAAUCAAUUGCUA
14 nt
3' ss
PPT
BP
DT-A
3'
2.PTM+SF-Py1:
5'—GC U AGC C UGGGA CAAGG ACA CUGC UUCACCCGGUUAGUAG ACCACAGCCCUGAGCC
GA CG - UCG A GCUCU UAUUA UGU CUUC AAGUGGGUCAAUCAUC AAAUAAUCAAUUGCUA
14 nt
3' ss
PPT
BP
DT-A
3'
3.PTM+SF-Py2:
5'—GC U AGC C UGGGACAAGGACA CU GCUUCACCCGGUUAGUAG ACCACAGCCCUGAGCC
GA CG - UCG A GCUCUGUUCCUGU CU UGAAGUGGGUCAAUCAUC AAAUAAUCAAUUGCUA
14 nt
3' ss
PPT
BP
DT-A
3'

FIG.4A

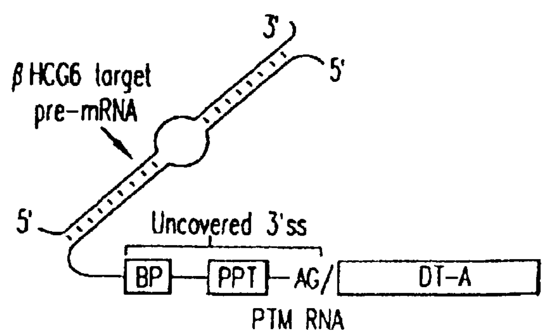

3'
5'
βHCG6 target
pre-mRNA
5'
Uncovered 3'ss
BP
PPT
AG/
DT-A
PTM RNA

FIG.4B

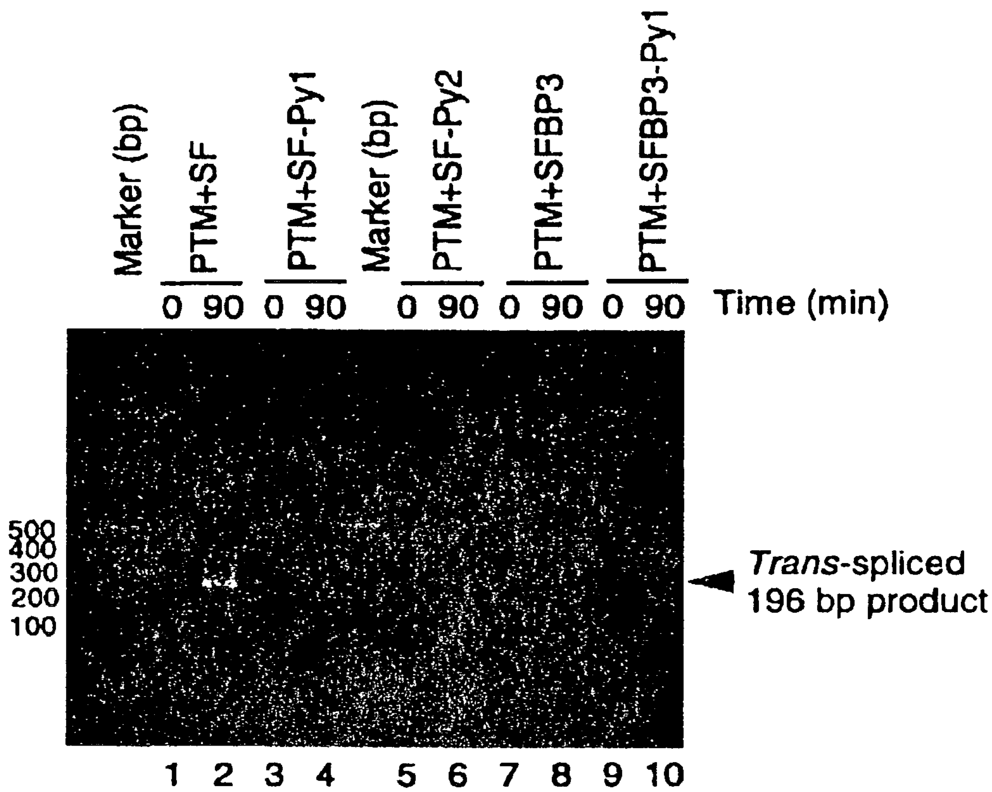

Marker (bp)
PTM+SF
PTM+SF-Py1
Marker (bp)
PTM+SF-Py2
PTM+SFBP3
PTM+SFBP3-Py1
0 90 0 90 0 90 0 90 0 90
Time (min)
500
400
300
200
100
Trans-spliced 196 bp product
1 2 3 4 5 6 7 8 9 10

FIG.4C

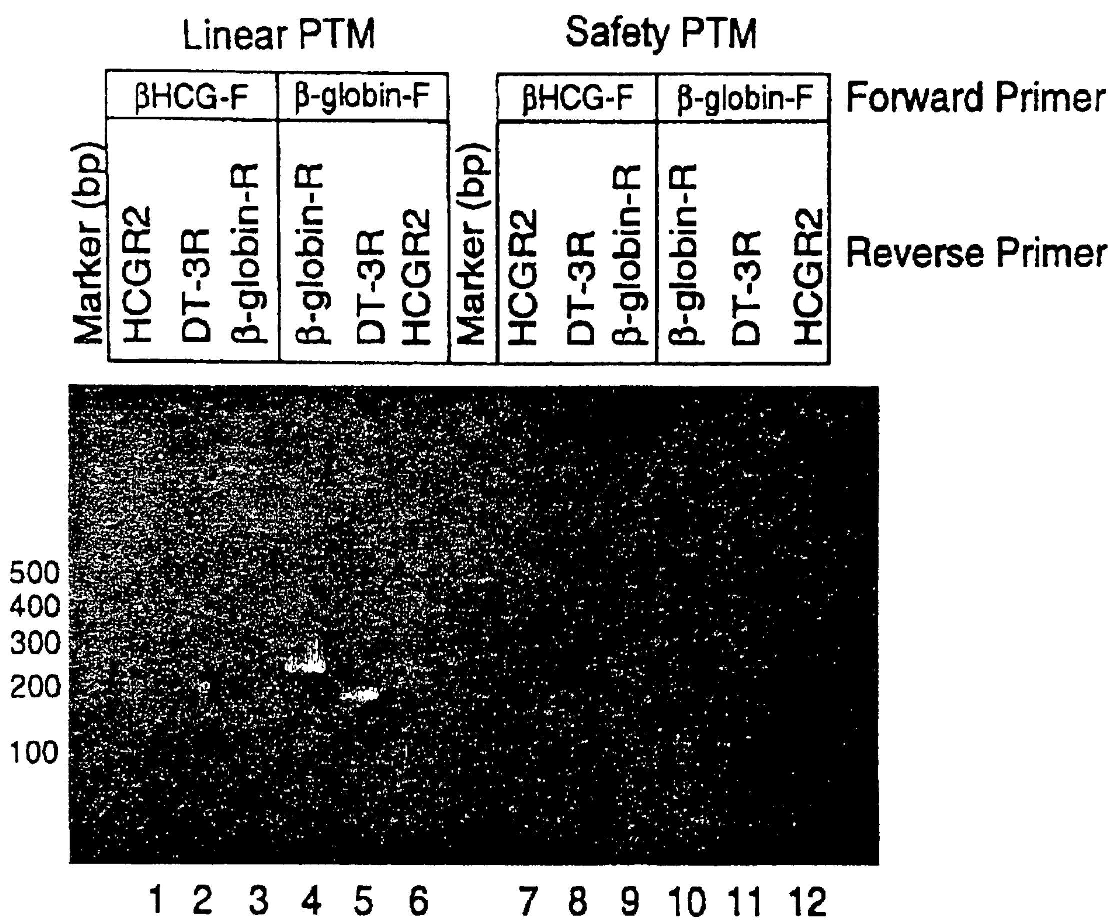

Linear PTM
Safety PTM
βHCG-F
β-globin-F
βHCG-F
β-globin-F
Forward Primer
Marker (bp)
HCGR2
DT-3R
β-globin-R
β-globin-R
DT-3R
HCGR2
Marker (bp)
HCGR2
DT-3R
β-globin-R
β-globin-R
DT-3R
HCGR2
Reverse Primer
500
400
300
200
100
1 2 3 4 5 6 7 8 9 10 11 12

FIG.5

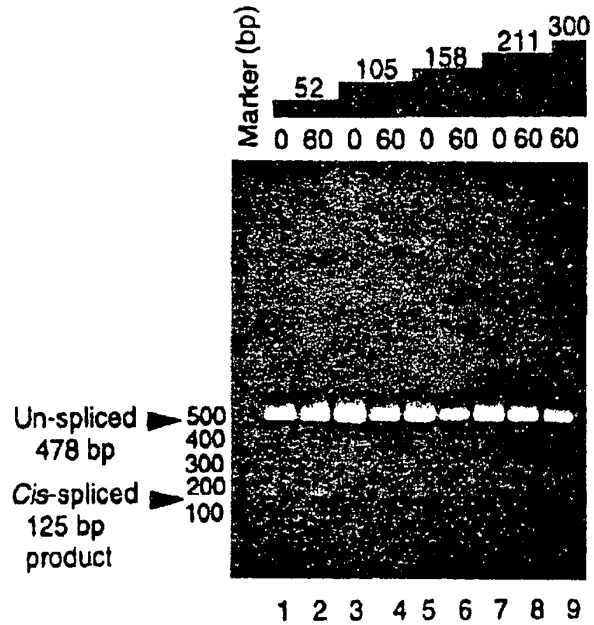

Marker (bp)
52
105
158
211
300
0 60 0 60 0 60 0 60 60
Un-spliced
478 bp
Cis-spliced
125 bp
product
500
400
300
200
100
1 2 3 4 5 6 7 8 9

FIG.6A

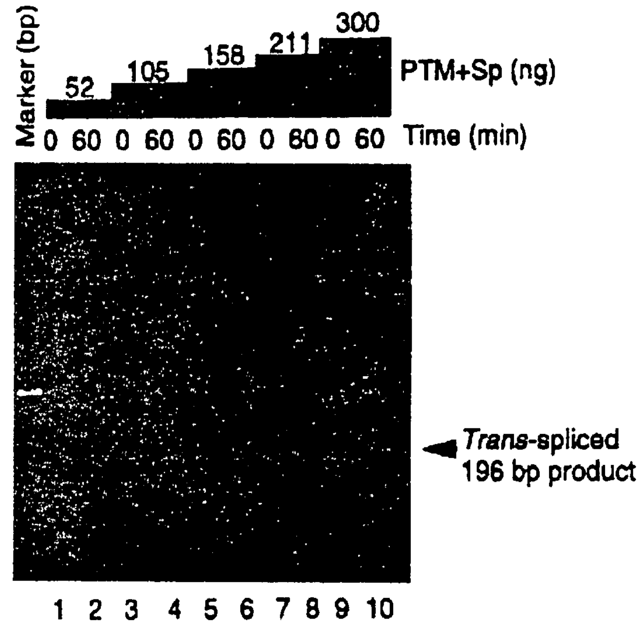

Marker (bp)
52
105
158
211
300
PTM+Sp (ng)
0 60 0 60 0 60 0 60 0 60
Time (min)
Trans-spliced
196 bp product
1 2 3 4 5 6 7 8 9 10

FIG.6B

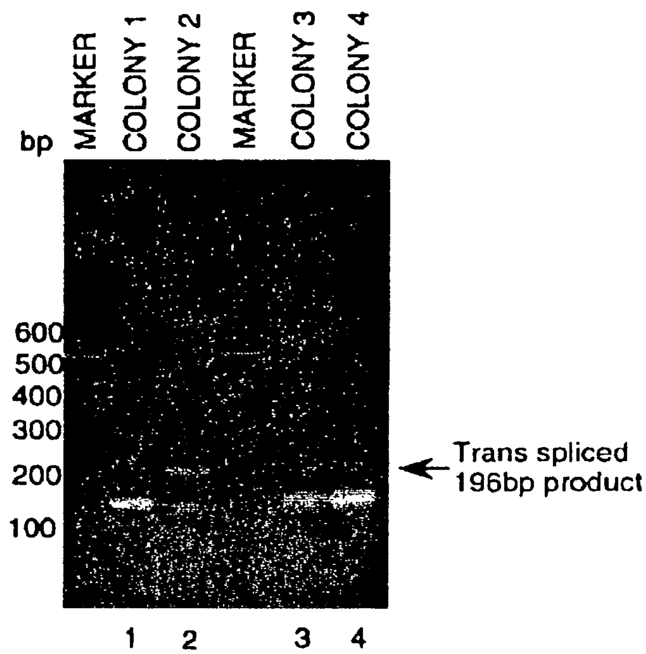

bp
MARKER
COLONY 1
COLONY 2
MARKER
COLONY 3
COLONY 4
600
500
400
300
200
100
Trans spliced
196bp product
1
2
3
4

FIG.7A

EXON 1 OF βHCG6 ↓

5'-CAGGGGACGCACCAAGGATGGAGATGTTCCAG-GGCGCTGATGATGTTGTT

↓1ST CODING NUCLEOTIDE OF DT-A

GATTCTTCTTAAATCTTTTGTGATGGAAAACTTTTCTTCGTACCACGGGACTA

AACCTGGTTATGTAGATTCCATTCAAAAA-3'

FIG.7B

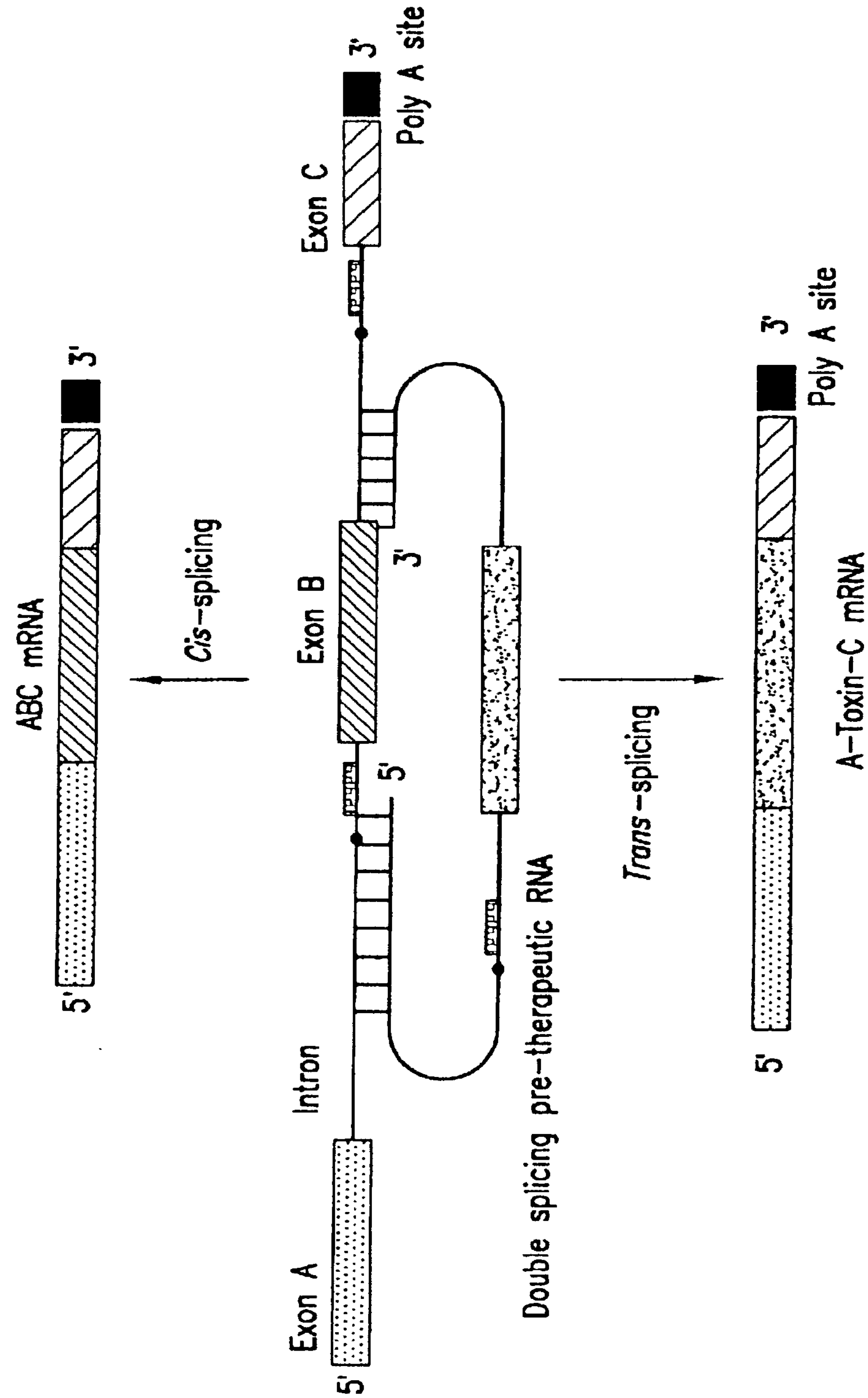
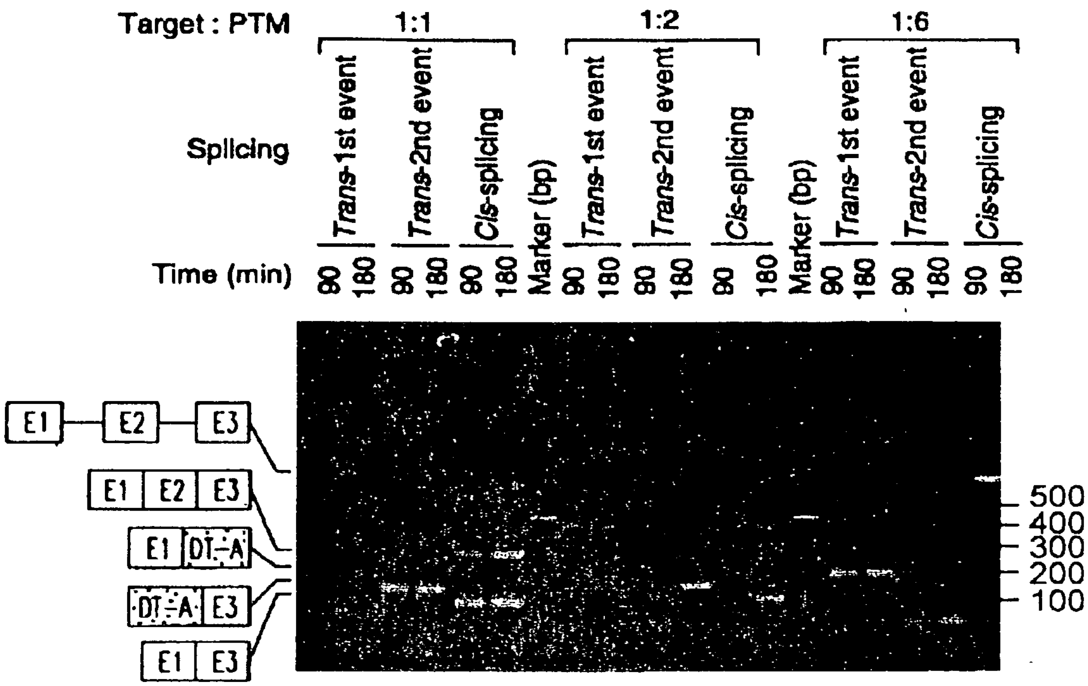

ABC mRNA
5'
3'
Cis-splicing
Exon A
5'
Intron
Exon B
5'
3'
Exon C
3'
Poly A site
Double splicing pre-therapeutic RNA
Trans-splicing
A-Toxin-C mRNA
5'
3'
Poly A site

FIG.8A



Cis- spliced products

E1 E2 E3 = NORMAL *cis*-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

Trans- spliced products

E1 DT-A = 1st EVENT, 196bp. *Trans*-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. *Trans*- SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

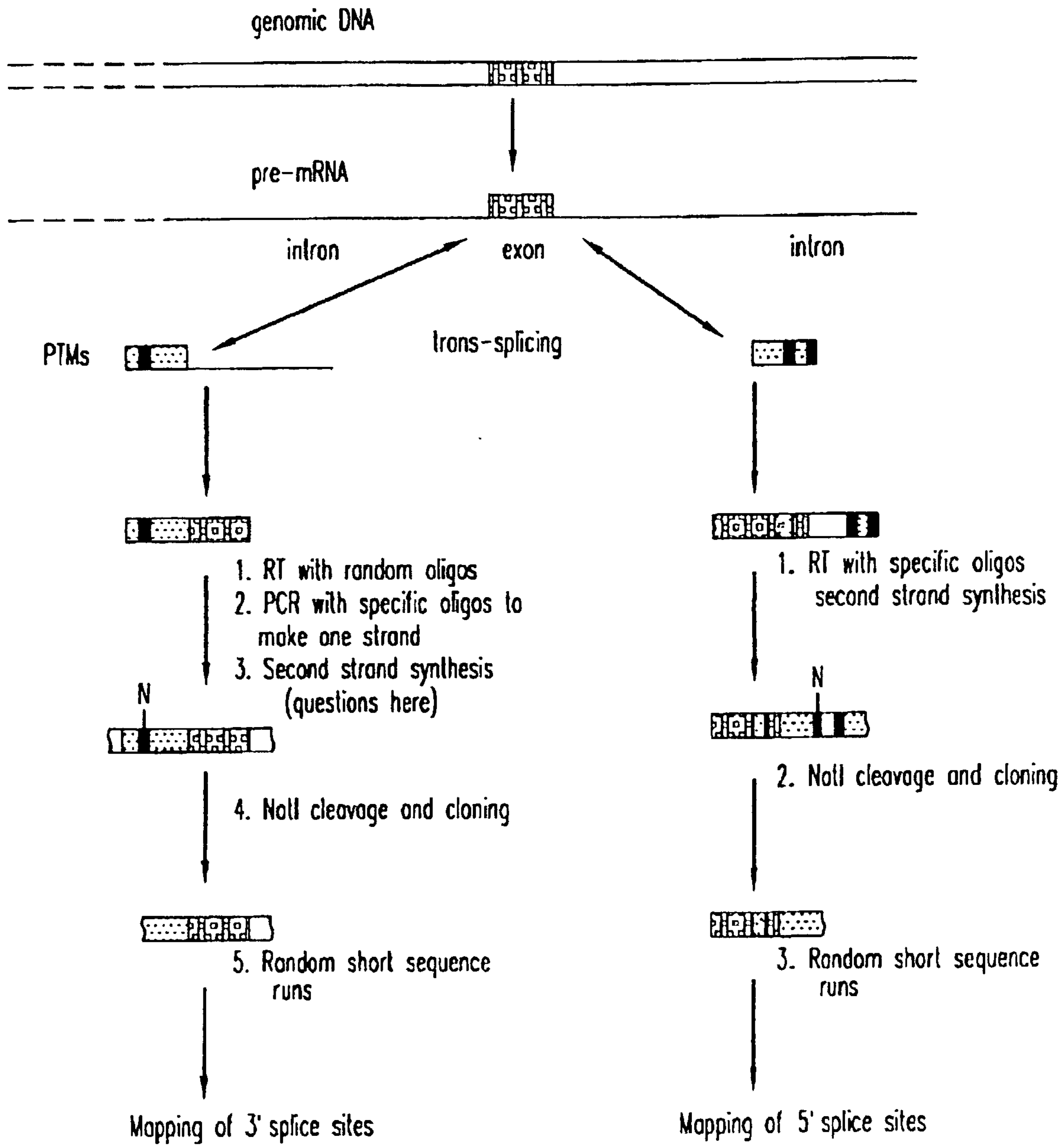

genomic DNA
pre-mRNA
intron
exon
intron
PTMs
trans-splicing
1. RT with random oligos
2. PCR with specific oligos to
make one strand
3. Second strand synthesis
(questions here)
N
4. NotI cleavage and cloning
5. Random short sequence
runs
Mapping of 3' splice sites
1. RT with specific oligos
second strand synthesis
N
2. NotI cleavage and cloning
3. Random short sequence
runs
Mapping of 5' splice sites

FIG.9

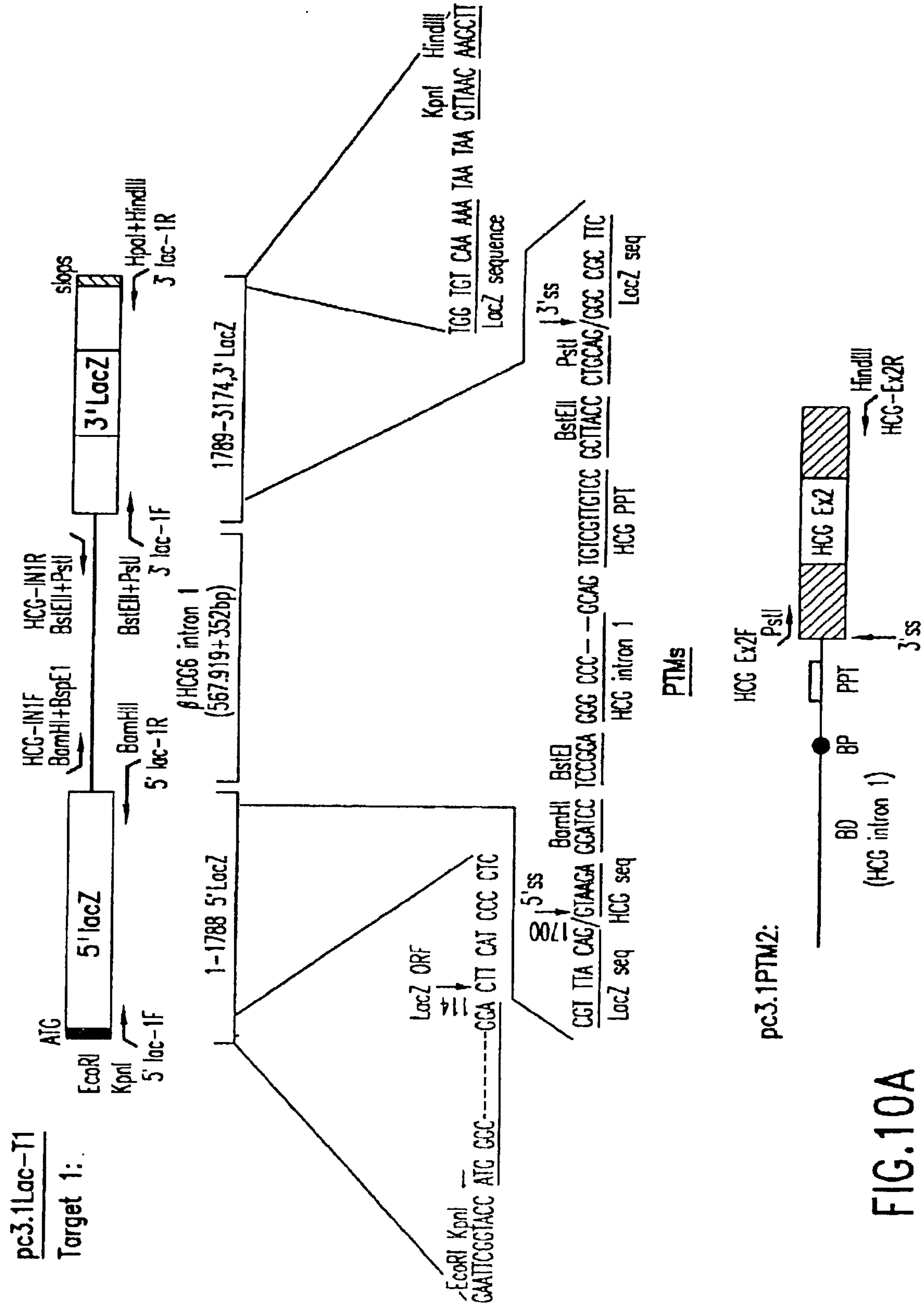

pc3.1Lac-T1
Target 1:
EcoRI
KpnI
ATG
5'lac-1F
5'lacZ
HCG-IN1F
BamHI+BspE1
BamHI
5'lac-1R
HCG-IN1R
BstEII+PstI
BstEII+PstI
3'lac-1F
3'LacZ
stops
HpaI+HindIII
3'lac-1R
1-1788 5'LacZ
βHCG6 intron 1
(567.919+352bp)
1789-3174,3'LacZ
EcoRI KpnI
GAATTCGGTACC ATG GGC
LacZ ORF
GGA CTT CAT CCC CTC
CGT TTA CAG/GTAAGA GGATCC TCCGGA GGG CCC- -GCAG TGTCGTGTCC GGTTACC CTGCAG/GGC CGC TTC
LacZ seq HCG seq LacZ seq HCG intron 1 HCG PPT LacZ seq
1788
5'ss
BamHI
BstEII
BstEII
PstI
3'ss
TGG TGT CAA AAA TAA TAA GTTAAC AAGCTT
LacZ sequence
KpnI
HindIII
PTMs
pc3.1PTM2:
BD
(HCG intron 1)
BP
PPT
HCG Ex2F
PstI
3'ss
HCG Ex2
HindIII
HCG-Ex2R

FIG.10A

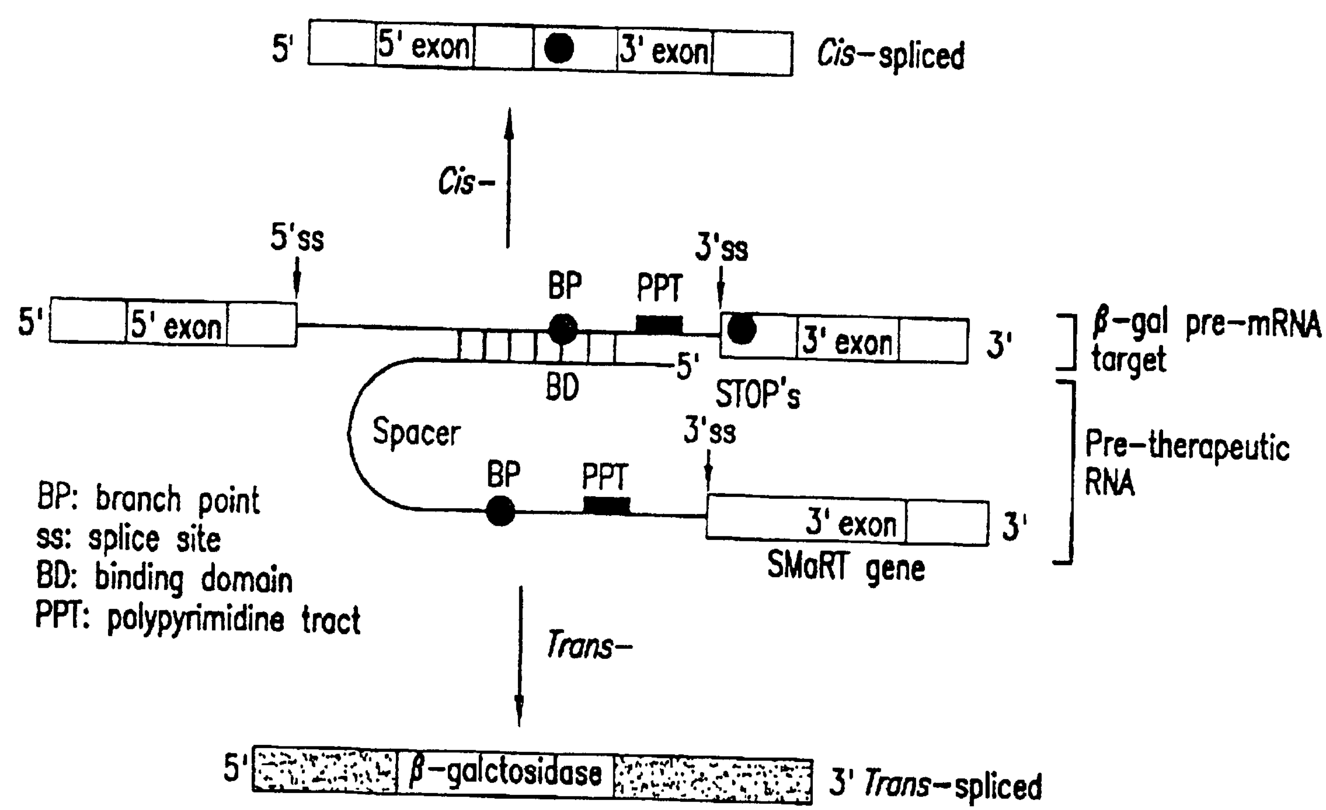

5'
5' exon
3' exon
Cis–spliced
Cis–
5'ss
3'ss
BP
PPT
3'
β–gal pre–mRNA target
BD
5'
STOP's
Spacer
Pre–therapeutic RNA
BP: branch point
ss: splice site
BD: binding domain
PPT: polypyrimidine tract
SMaRT gene
Trans–
β–galctosidase
3' Trans–spliced

FIG.10B

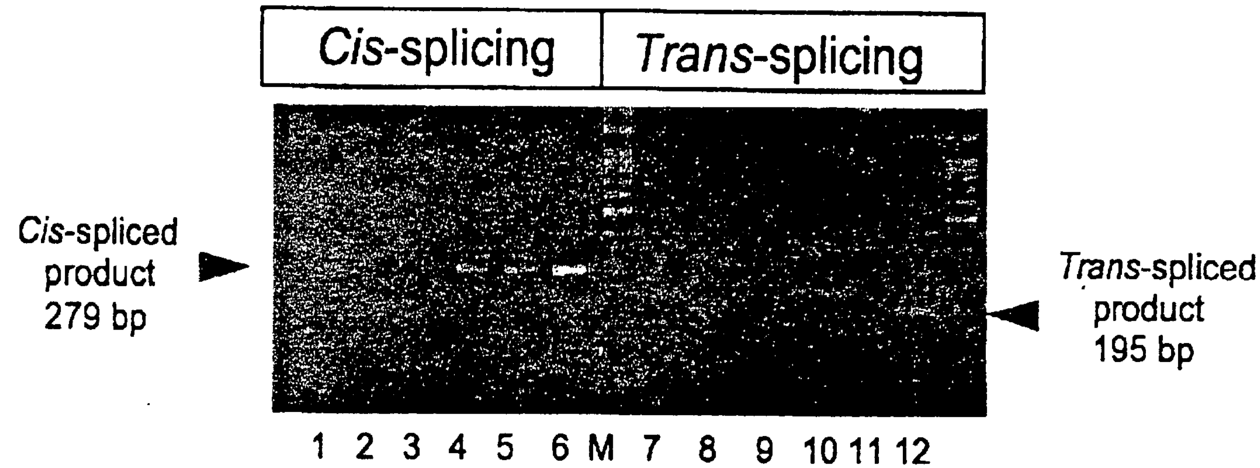

Cis-splicing
Trans-splicing
Cis-spliced product 279 bp
Trans-spliced product 195 bp
1 2 3 4 5 6 M 7 8 9 10 11 12

FIG.11A

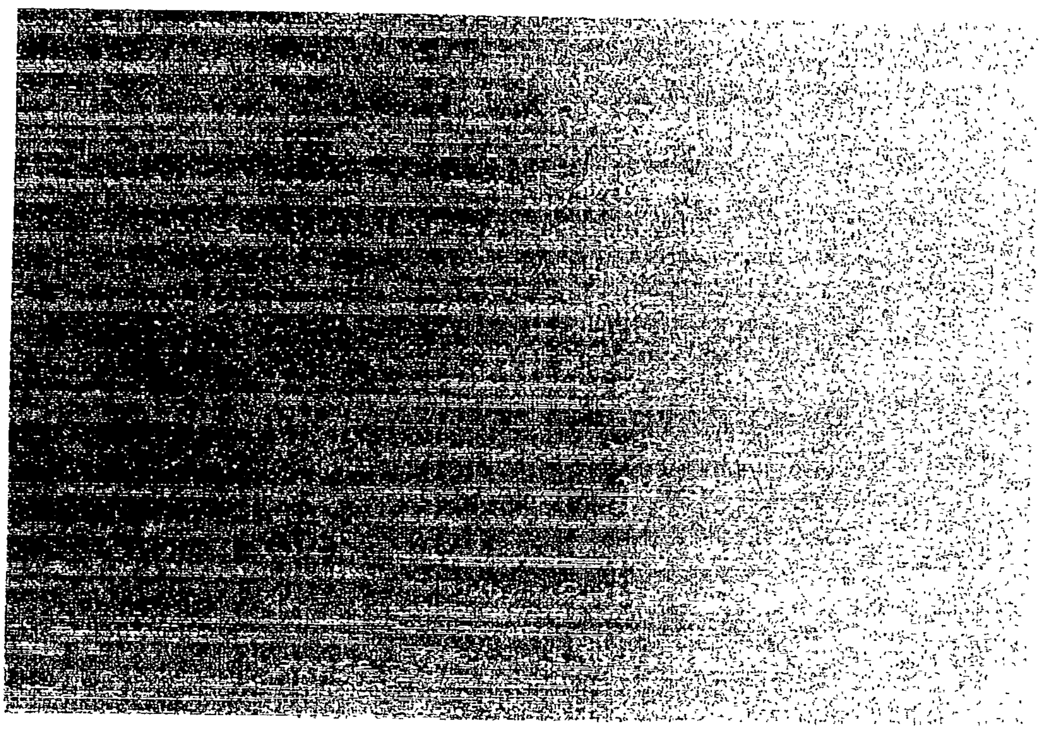


FIG.11B

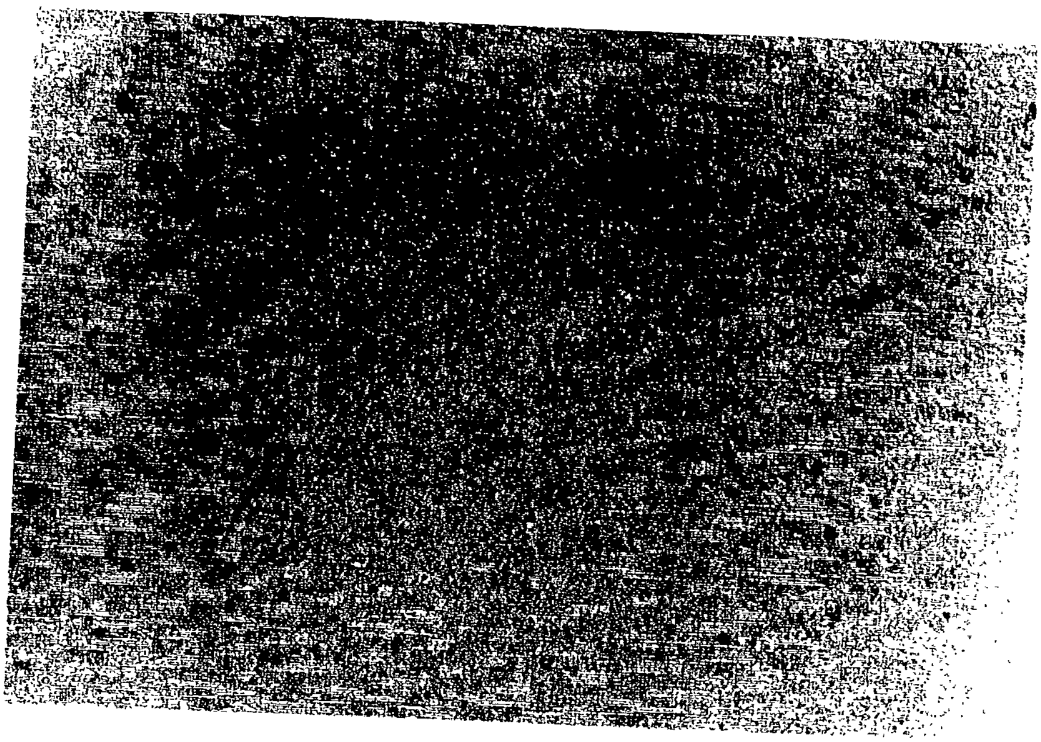


FIG. 11C

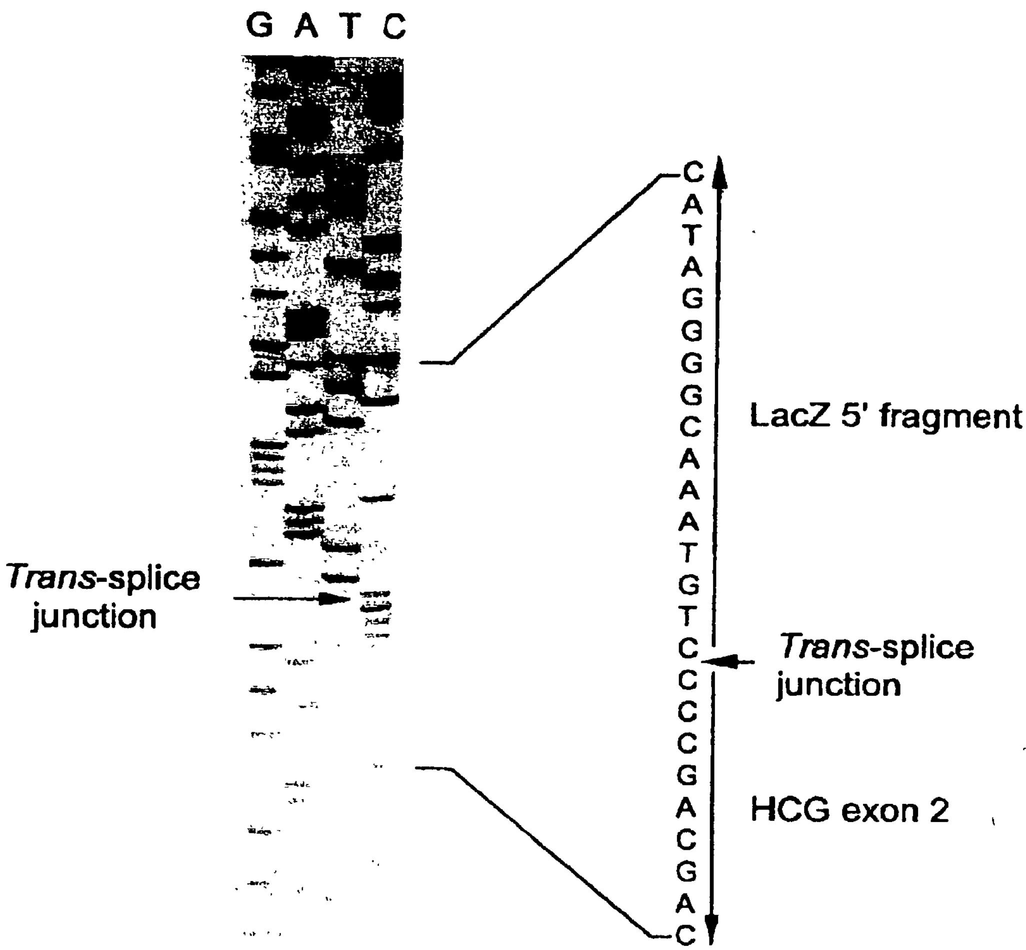

G A T C
C
A
T
A
G
G
G
G
C
A
A
A
T
G
T
C
C
C
C
G
A
C
G
A
C
LacZ 5' fragment
Trans-splice junction
Trans-splice junction
HCG exon 2

FIG.12A

1. NUCLEOTIDE SEQUENCES OF THE *cis*-SPLICED PRODUCT (285 bp):

BioLac-TR1

<u>GGCTTTCGCTACCTGGAGAG</u>ACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTG

Splice junction

GCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGCGGCTTCGTCTAATAATG

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTGGTCGGCTTACGGCGGTGATTT

Lac-TR2

TGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGAC<u>CGCACGCCGCATCCAG</u>

2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)

BioLac-TR1

<u>GGCTTTCGCTACCTGGAGAG</u>ACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGG

Splice junction

CGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGGCTGCTGCTGTTGCTGCTGCT

HCGR2

GAGCATGGGCGGGACATGGGCATCCAAGGAG<u>CCACTTCGGCCACGGTGCCG</u>

FIG.12B

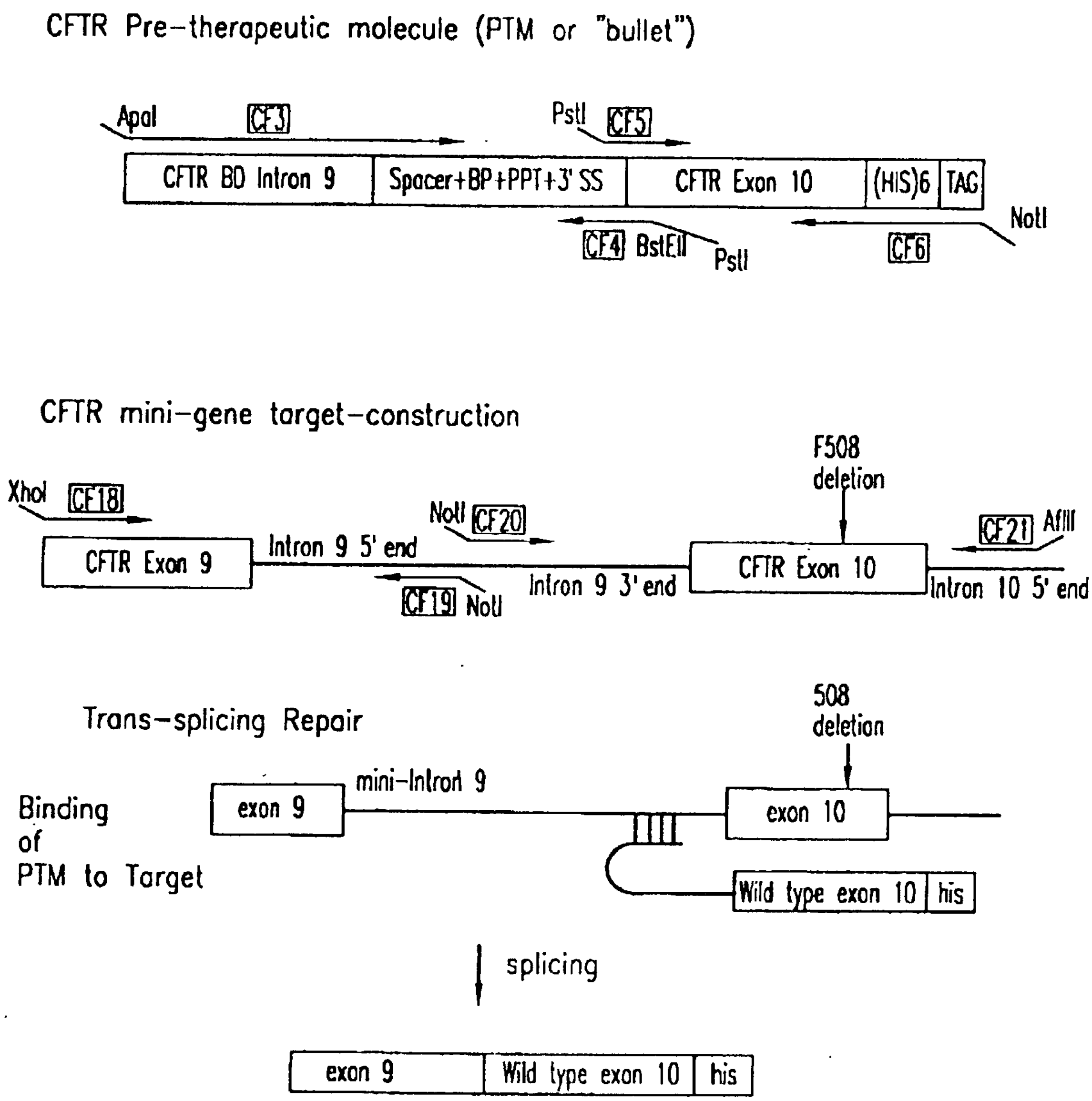

CFTR Pre-therapeutic molecule (PTM or "bullet")
ApaI
CF3
PstI
CF5
CFTR BD Intron 9
Spacer+BP+PPT+3' SS
CFTR Exon 10
(HIS)6
TAG
NotI
CF4
BstEII
PstI
CF6
CFTR mini-gene target-construction
F508
deletion
XhoI
CF18
NotI
CF20
CF21
AflII
CFTR Exon 9
Intron 9 5' end
CF19
NotI
Intron 9 3' end
CFTR Exon 10
Intron 10 5' end
Trans-splicing Repair
508
deletion
Binding
of
PTM to Target
exon 9
mini-Intron 9
exon 10
Wild type exon 10
his
splicing
exon 9
Wild type exon 10
his

FIG.13

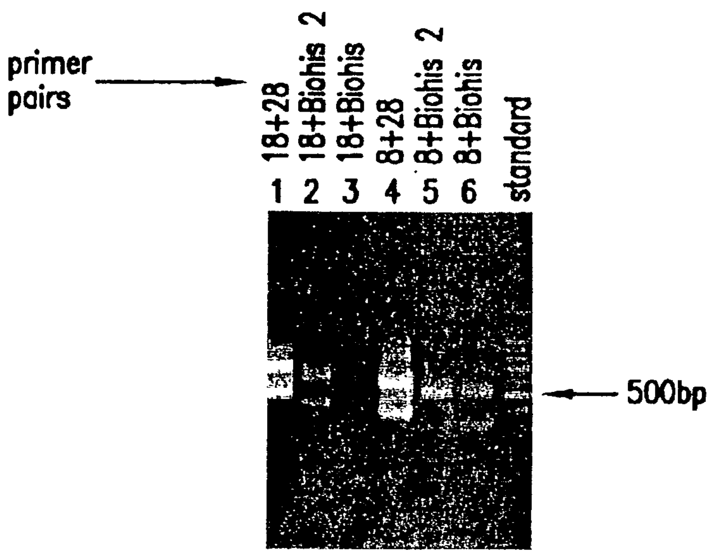

primer pairs
1 18+28
2 18+Biohis 2
3 18+Biohis
4 8+28
5 8+Biohis 2
6 8+Biohis
standard
500bp

FIG. 14

DNA sequence 500 b.p. GCTAGCGTTTAA ... TGCCACTCCCAC linear

Positions of Restriction Endonucleases sites (unique sites underlined)

```
                Sau96 I
                Hae III
                Sau96 I
                Ban II                  BINDING DOMAIN
Nhe I  Dra I    Apa I    INTRON 9 BD    Sac II                       Xho I  Sca I
GCTAGCGTTTAAACGGGCCCACCCATCATTATTAGGTCATTATCCGCGGAACATTATTATAACCTTGCTCGAGTACTAAC 80
CGATCGCAAATTTGCCCGGGTGGGTAGTAATAATCCAGTAATAGGCGCCTTGTAATAATATTGCAACGAGCTCATGATTG
1      8        15                            44                        68
                15                                                          72
                15
                16
                16

Kpn I                 Pst I 3'ss                 EXON 10 CFTR + HIS TAG + STOP
TGGTACCTCTTCTTTTTTTTCCTGCAGACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAAT 160
ACCATGGAGAAGAAAAAAAAGGACGTCTGAAGTGAAGATTACTACTAATACCCTCTTGACCTCGGAAGTCTCCCATTTTA
82                    102

         Xmn I           Dde I                                                F508
TAAGCACAGTGCAAGAATTTCATTCTGTTCTCAGTTTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTG 240
ATTCGTGTCACCTTCTTAAAGTAAGACAAGAGTCAAAAGGACCTAATACGGACCGTGGTAATTTCTTTTATAGTAGAAAC
         172             190

                                        Sph I                      HIS          STOP
GTGTTTCCTATGATGAATATAGATACAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGCATCATCATCATCATCATTAG 320
CACAAAGGATACTACTTATATCTATGTCTTCGCAGTAGTTTCGTACGGTTGATCTTCTCGTAGTAGTAGTAGTAGTAATC
                                        282
```

FIG.15A

```
                                                          Sac I
                                                          Ban II
                                                       Sau3A I
 Hae III                                               Dpn I                    HinD III
Not I            Pst I                                 BamH I         Kpn I                 Dra I
           EcoR V      EcoR I
GCGGCCGCCACTGTGCTGGATATCTGCAGAATTCCACCACACTGGACTAGTGGATCCGAGCTCGGTACCAAGCTTAAGTT 400
CGCCGGCGGTGACACGACCTATAGACGTCTTAAGGTGGTGTGACCTGATCACCTAGGCTCGAGCCATGGTTCGAATTCAA
321        339         349              CF28   372         384        399
 323           344                                  373           390
                                                    373
                                                      378           PRESENT IN PTM 3'UT
                                                      378              BUT NOT TARGET

    Sau3A I
    Dpn I
TAAACCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACC 480
ATTTGGCGACTAGTCGGAGCTGACACGGAAGATCAACGGTCGGTAGACAACAAACGGGGAGGGGGCACGGAAGGAACTGG
    410            CF27
    410

CTGGAAGGTGCCACTCCCAC 500
GACCTTCCACGGTGAGGGTG
```

Restriction Endonucleases site usage

Acc I	–	EcoR I	1	Nde I	–	Sau96 I	2
Apa I	1	EcoR V	1	Nhe I	1	Sca I	1
ApaL I	–	Hae II	–	Not I	1	Sma I	–
Avr II	–	Hae III	2	PflM I	–	Sph I	1
BamH I	1	HinC II	–	Pst I	2	Spl I	–
Ban II	2	HinD III	1	Pvu I	–	Ssp I	–
Bbe I	–	Hinf I	–	Pvu II	–	Stu I	–

FIG.15B

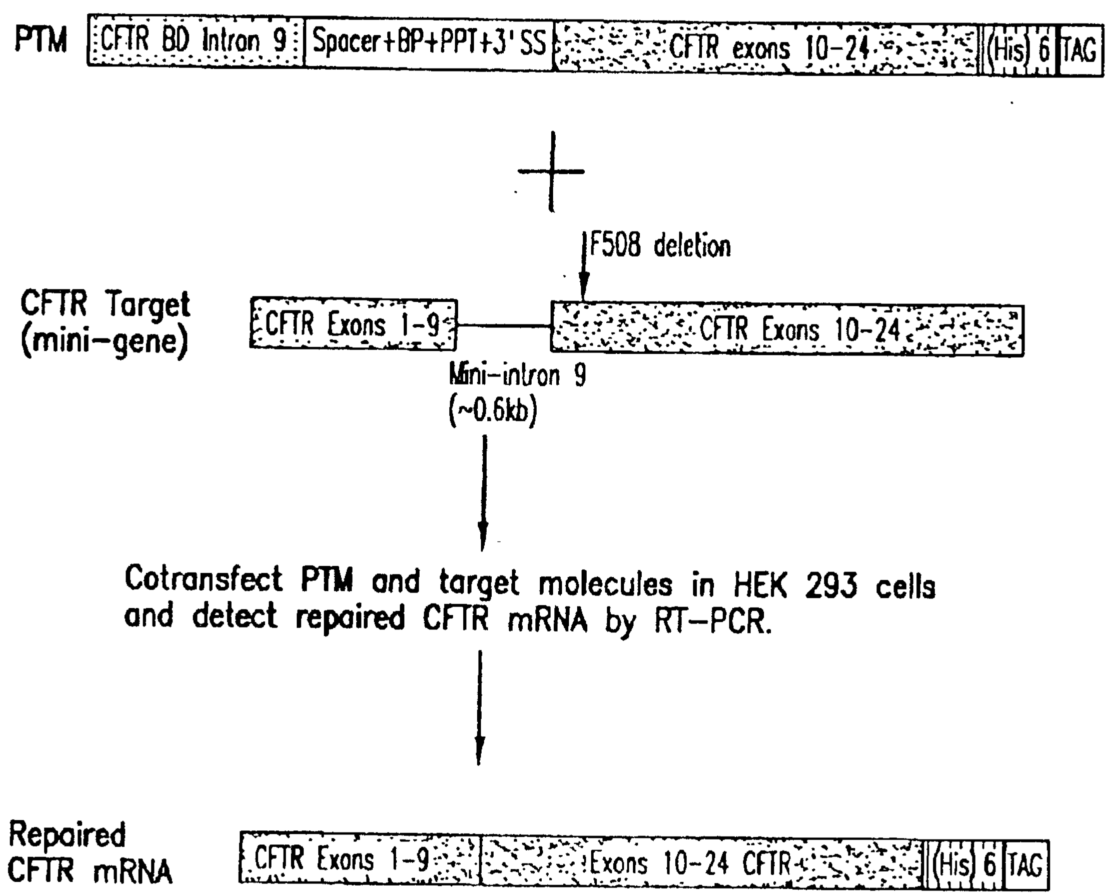

PTM
CFTR BD Intron 9
Spacer+BP+PPT+3' SS
CFTR exons 10-24
(His) 6
TAG
+
F508 deletion
CFTR Target
(mini-gene)
CFTR Exons 1-9
CFTR Exons 10-24
Mini-intron 9
(~0.6kb)
Cotransfect PTM and target molecules in HEK 293 cells
and detect repaired CFTR mRNA by RT-PCR.
Repaired
CFTR mRNA
CFTR Exons 1-9
Exons 10-24 CFTR
(His) 6
TAG

FIG.16

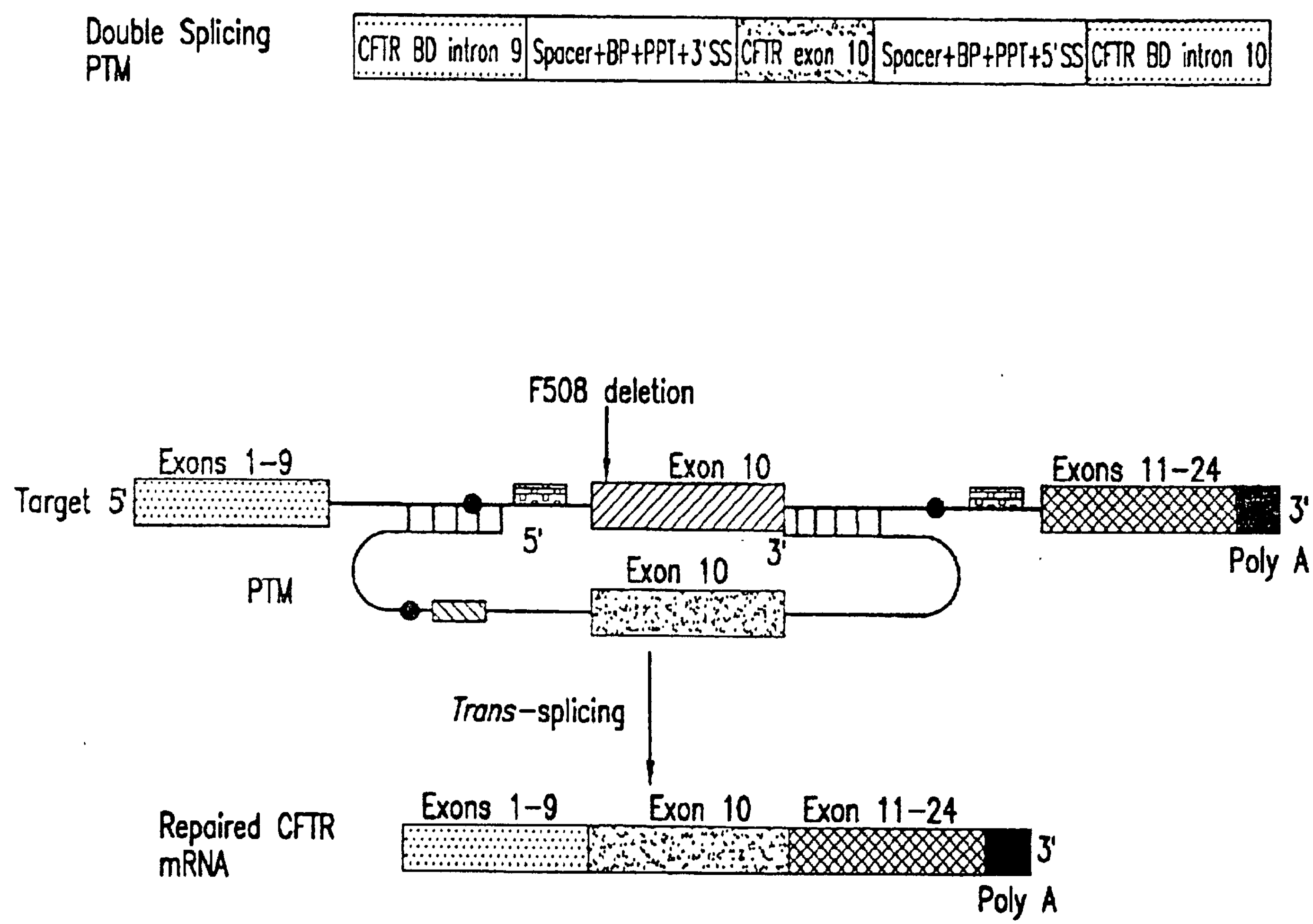

Double Splicing PTM
CFTR BD intron 9
Spacer+BP+PPT+3'SS
CFTR exon 10
Spacer+BP+PPT+5'SS
CFTR BD intron 10
F508 deletion
Target 5'
Exons 1-9
Exon 10
Exons 11-24
5'
3'
3'
Poly A
PTM
Exon 10
Trans-splicing
Repaired CFTR mRNA
Exons 1-9
Exon 10
Exon 11-24
3'
Poly A

FIG.17

DOUBLE *TRANS*-SPLICING SPECIFIC TARGET

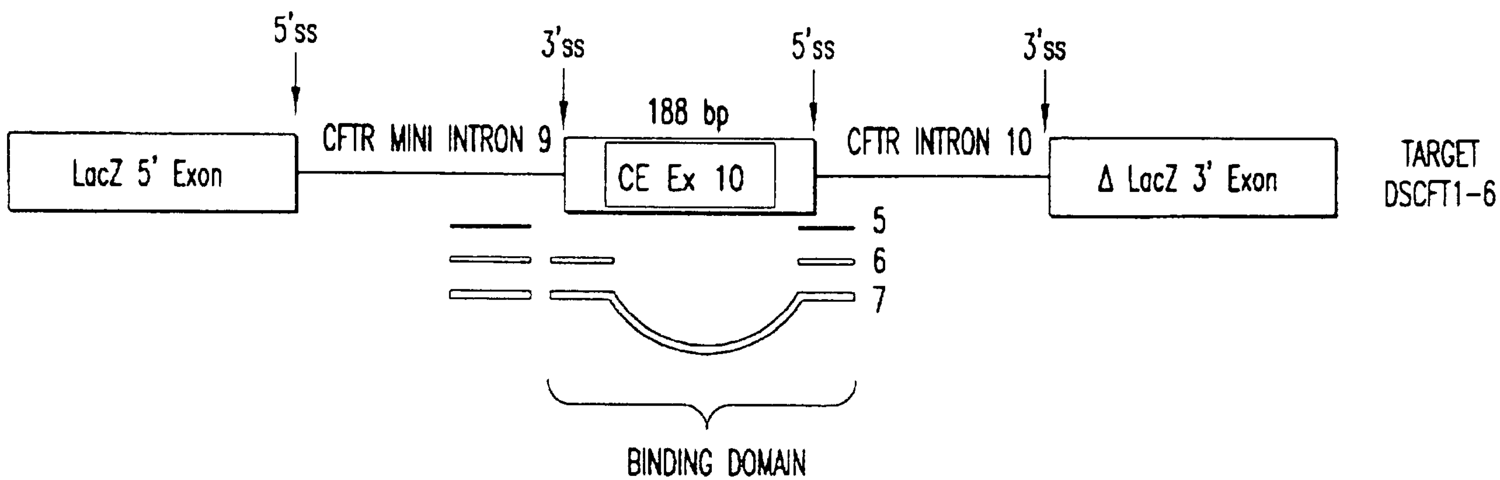


FIG.18

DOUBLE *TRANS*-SPLICING PTMs

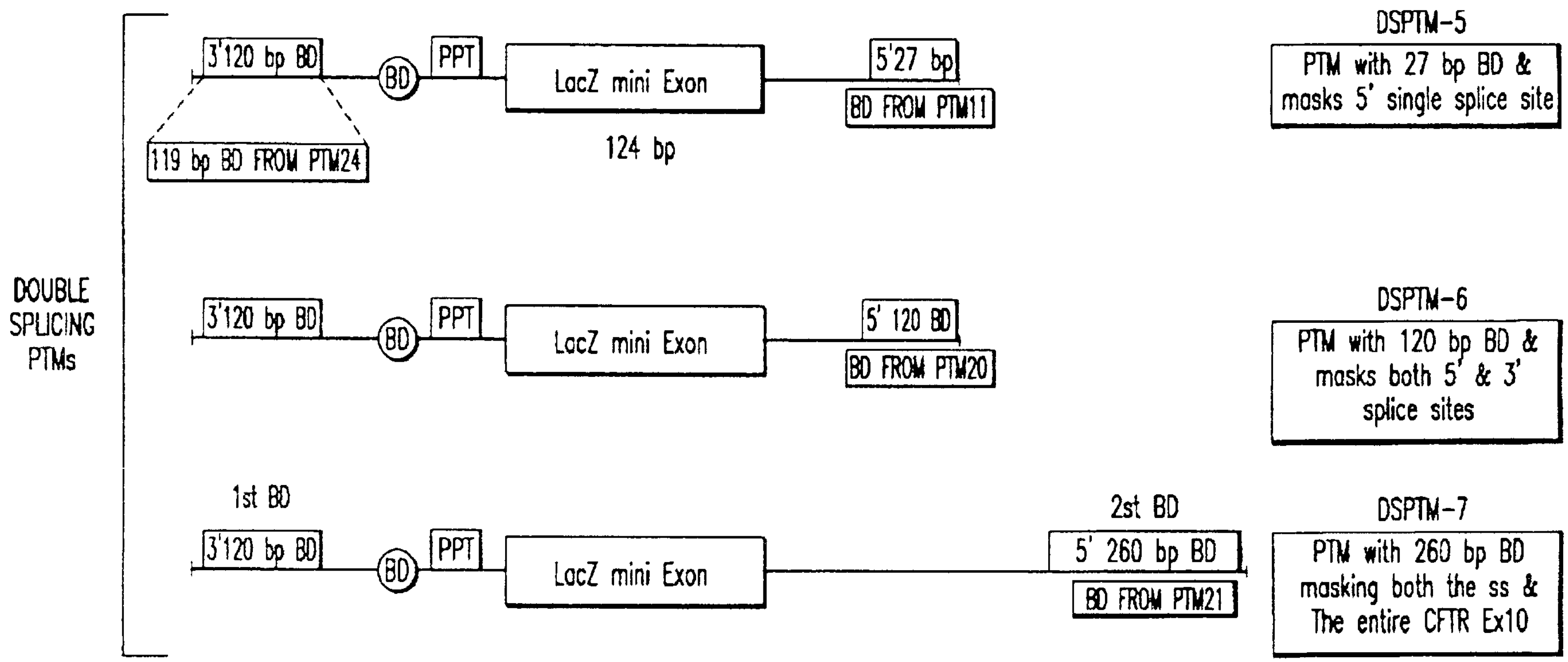


FIG.19

DOUBLE *TRANS*-SPLICING β-GAL MODEL

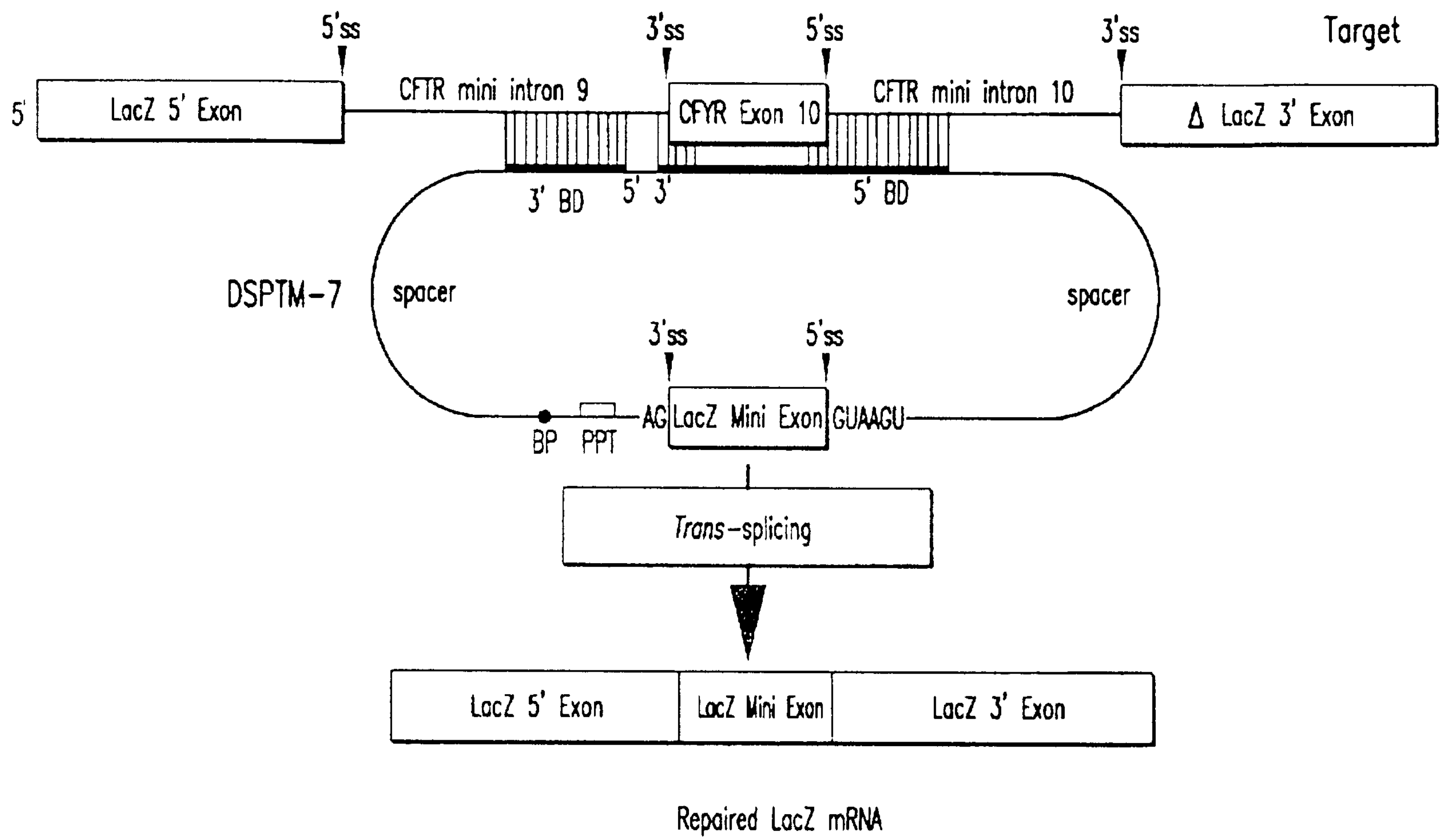
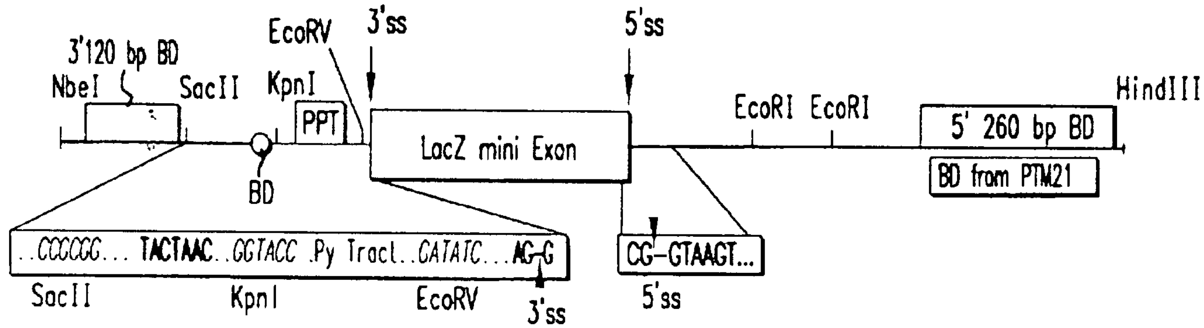


FIG.20



(1) 3' BD (120 BP): GATTCACTTGCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTATTTGTAAAGATTCTATTAACTCATTTGATTC
AAAATATTTAAAATACTTCCTGTTTCATACTCTGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATTATAACGTTGCTCGAA

(3) Branch point, pyrimidine tract and acceptor splice site: BP TACTAAC T Kpn I *GGTACC* PPT TCTTCTTTTTTTTTT EcoRV *GATATC* CTGCAG 3'ss GGC GGC (LacZ mini exon)

(4) 5' donor site and 2nd spacer sequence: | TGA ACG (LacZ mini exon) 5'ss *GTAAGT* GTTATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACG
CTAAGATCCACCGG

(5) 5' BD (260 BP): TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTT*GAATTC*ATGCTTTGATGACGCTTCTGTATCTATATTCATCATTGGAA
ACACCAATGATTTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGTGCTTAA
AAAAACCCTCT*GAATTC*TCCATTTCTCCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATTATTAACTCA
TTATCAAATCACGC

FIG.21

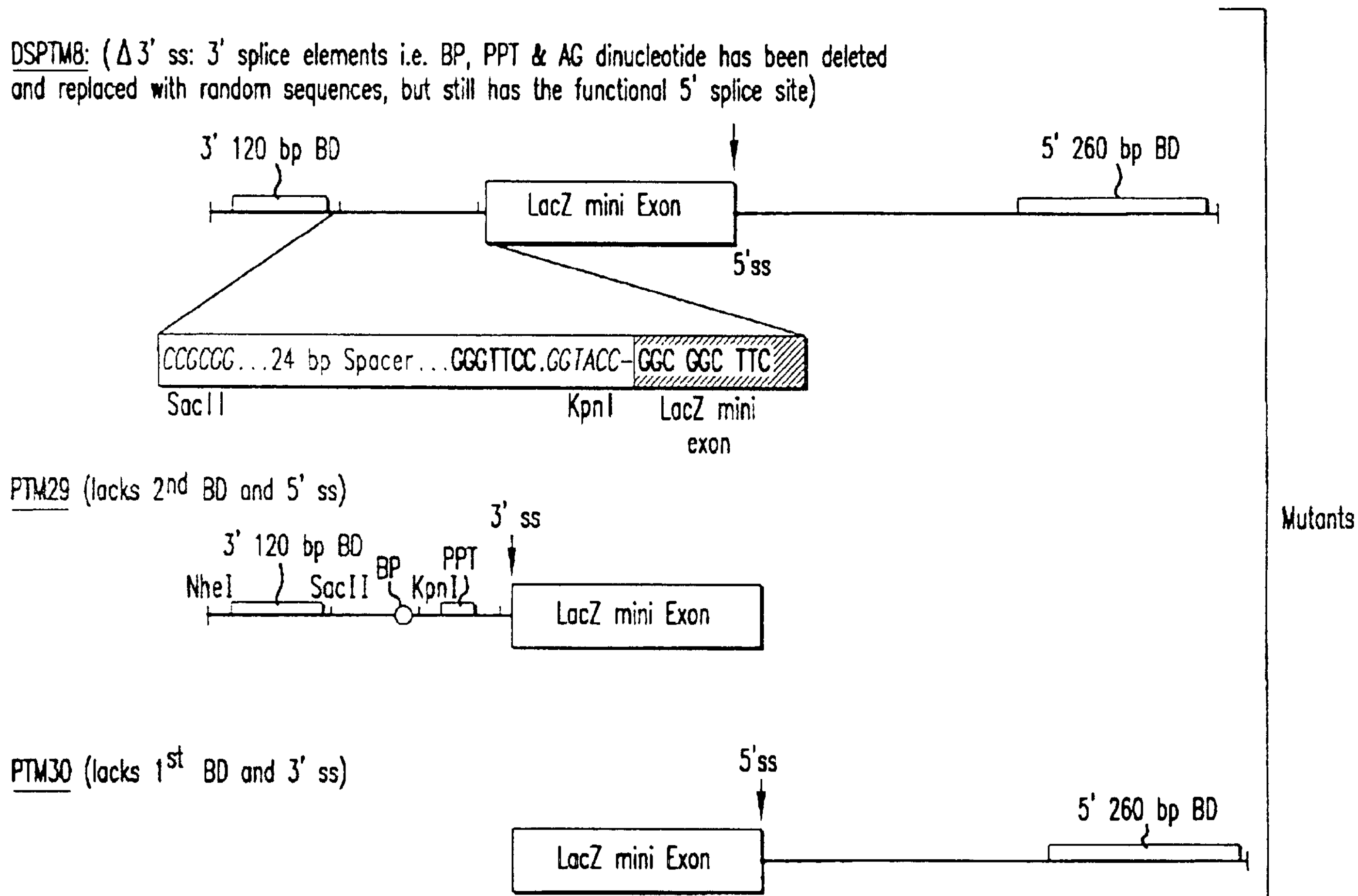

DSPTM8: (Δ3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)
3' 120 bp BD
LacZ mini Exon
5'ss
5' 260 bp BD
CCGCGG...24 bp Spacer...GGGTTCC.GGTACC-GGC GGC TTC
SacII
KpnI
LacZ mini exon
PTM29 (lacks 2nd BD and 5' ss)
3' 120 bp BD
NheI
SacII
BP
KpnI
PPT
3' ss
LacZ mini Exon
PTM30 (lacks 1st BD and 3' ss)
5'ss
LacZ mini Exon
5' 260 bp BD
Mutants

FIG.22

ACCURACY OF DOUBLE *TRANS*-SPLICING REACTION

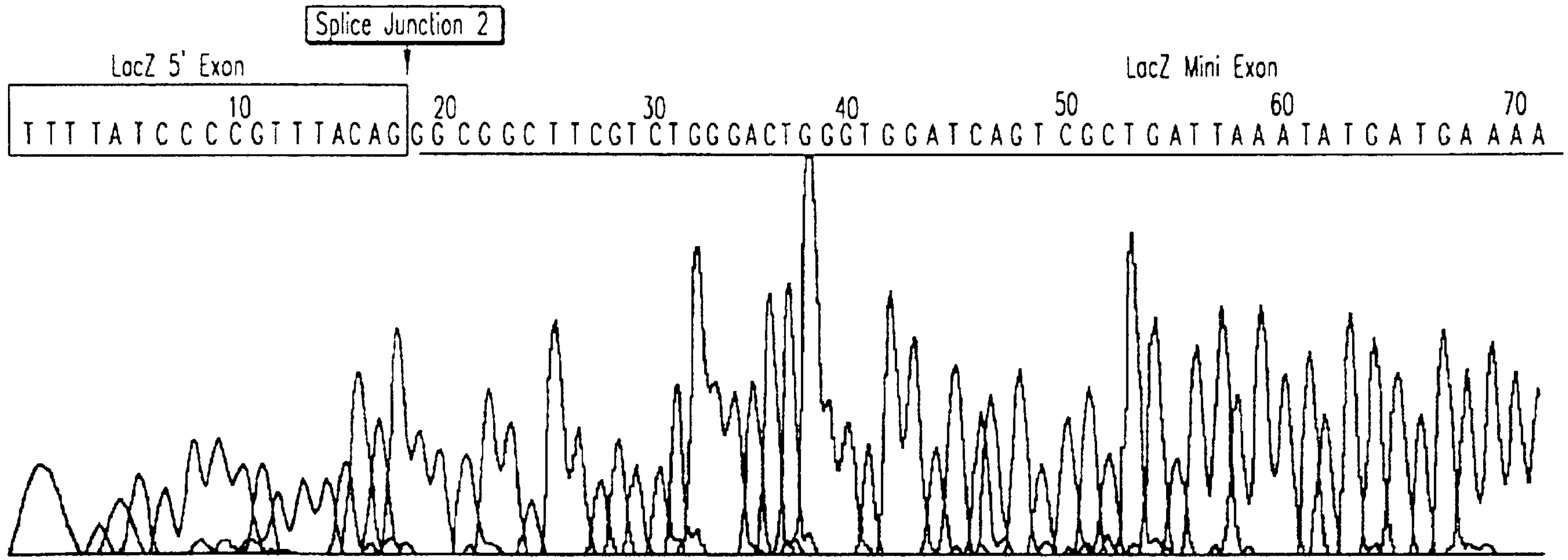


FIG.23A

ACCURACY OF DOUBLE *TRANS*-SPLICING REACTION

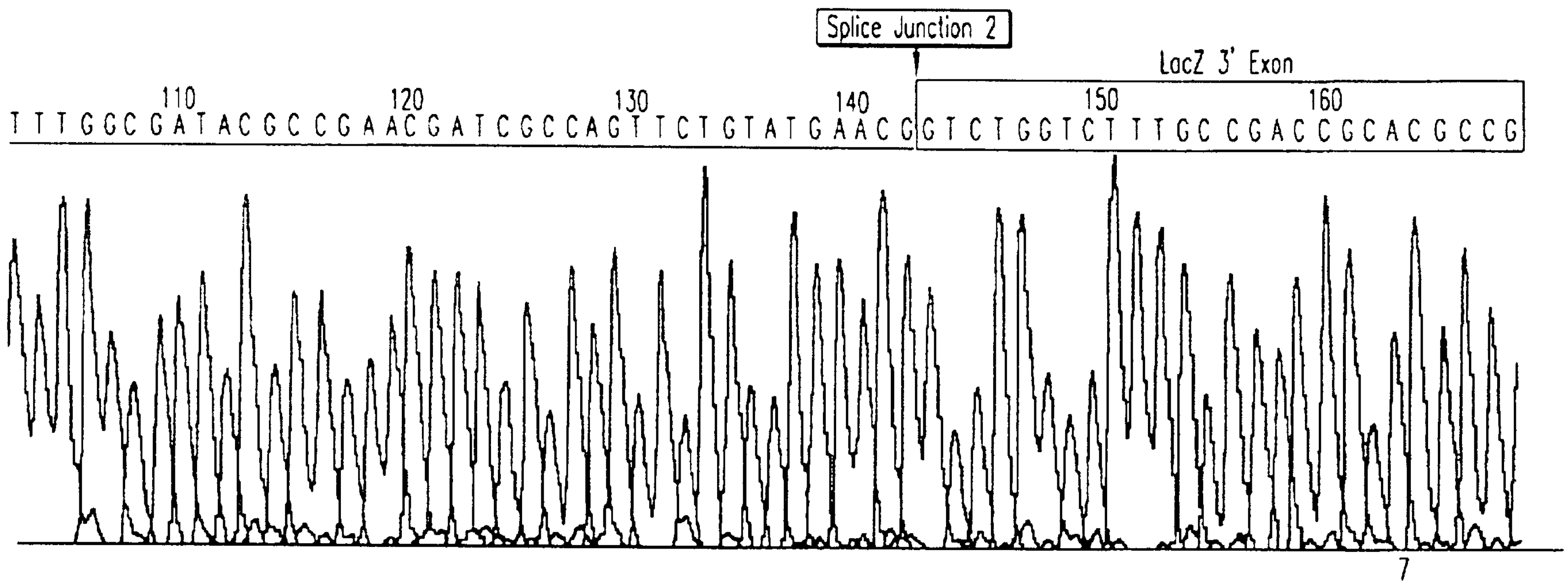
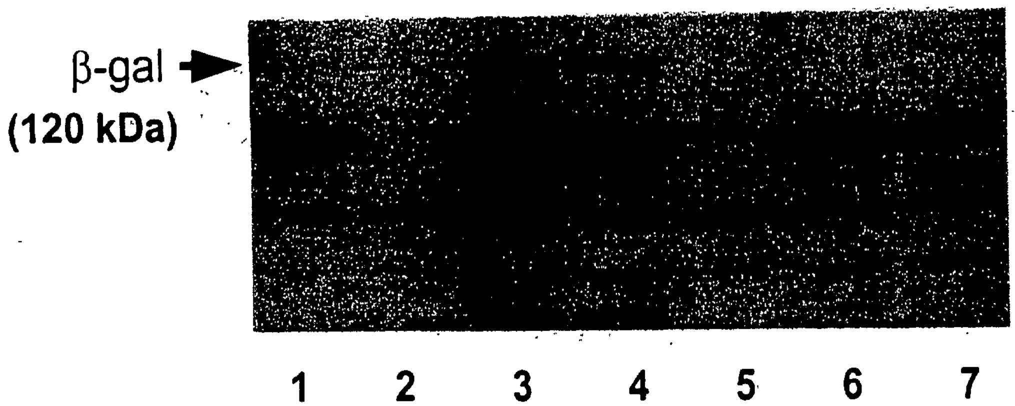


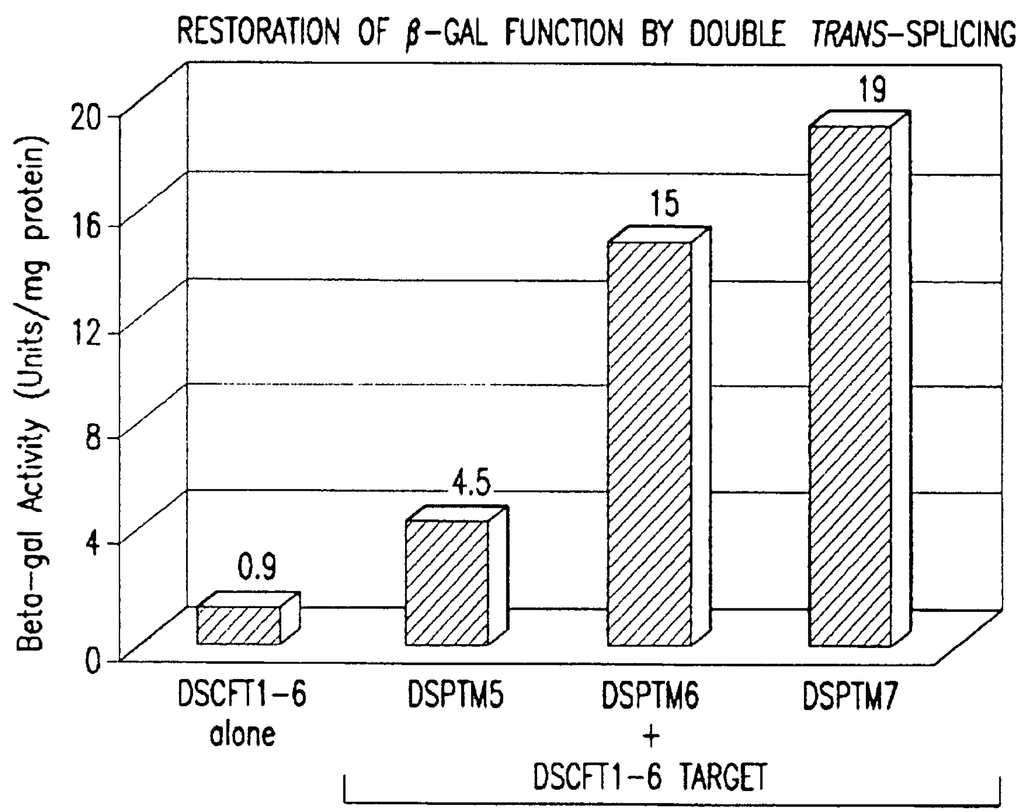
FIG.23B

Double *Trans*-splicing Produces Full-length Protein



Lane 1: DSCFT1.6 Target alone	25 μg
Lane 2: DSPTM7	25 μg
Lane 3 Target + PTM #6	25 μg
Lane 4: Target + PTM #9	25 μg
Lane 5: Delta 3' splice mutant alone	25 μg
Lane 6: Target + Delta 3' ss	25 μg
Lane 7: Target+PTM29+30 (mutants)	25 μg

Figure 24



Beta-gal Activity above
the Background level

DSPTM5: 5 fold
DSPTM6: 17 fold
DSPTM7: 21 fold

FIG.25

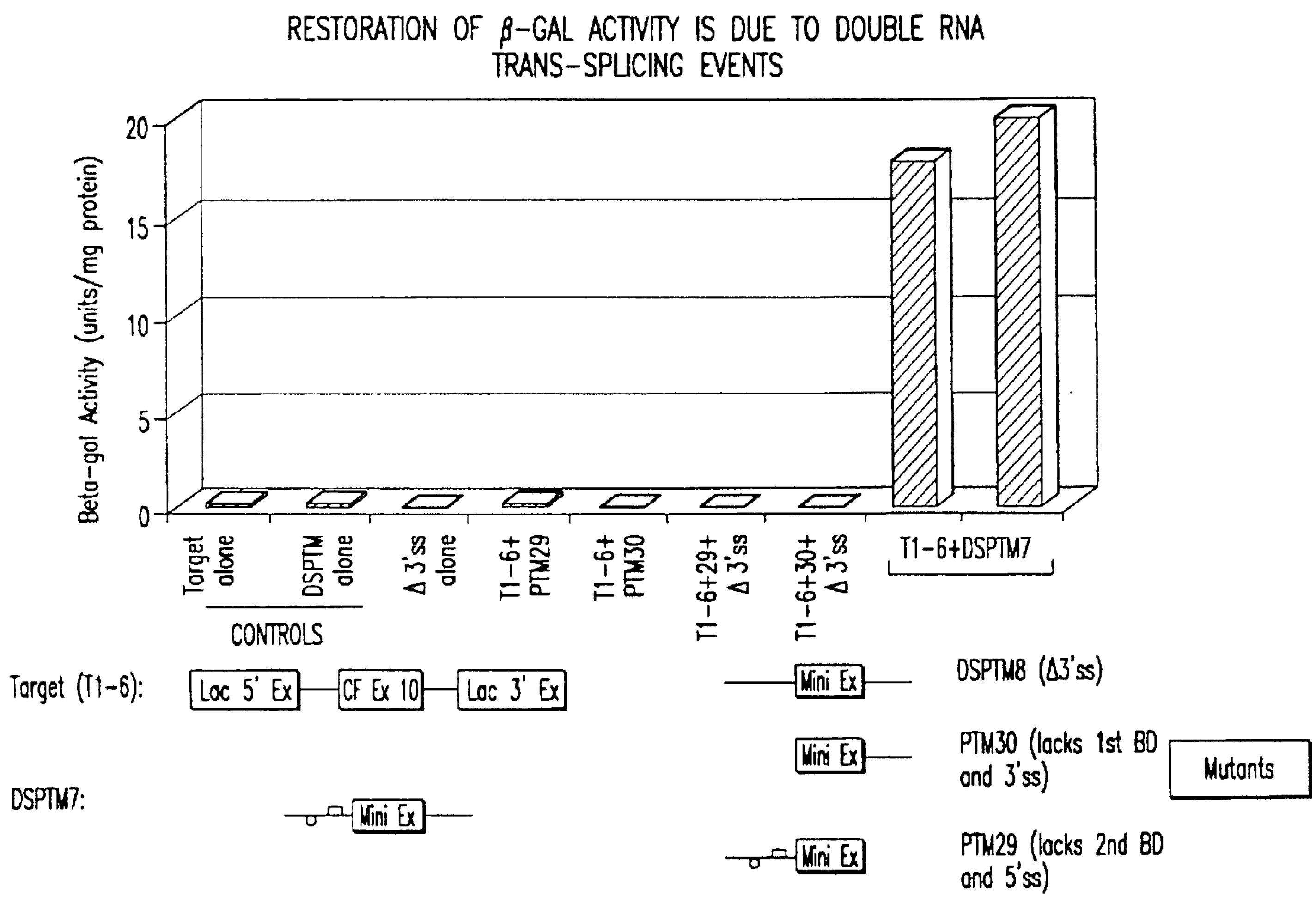
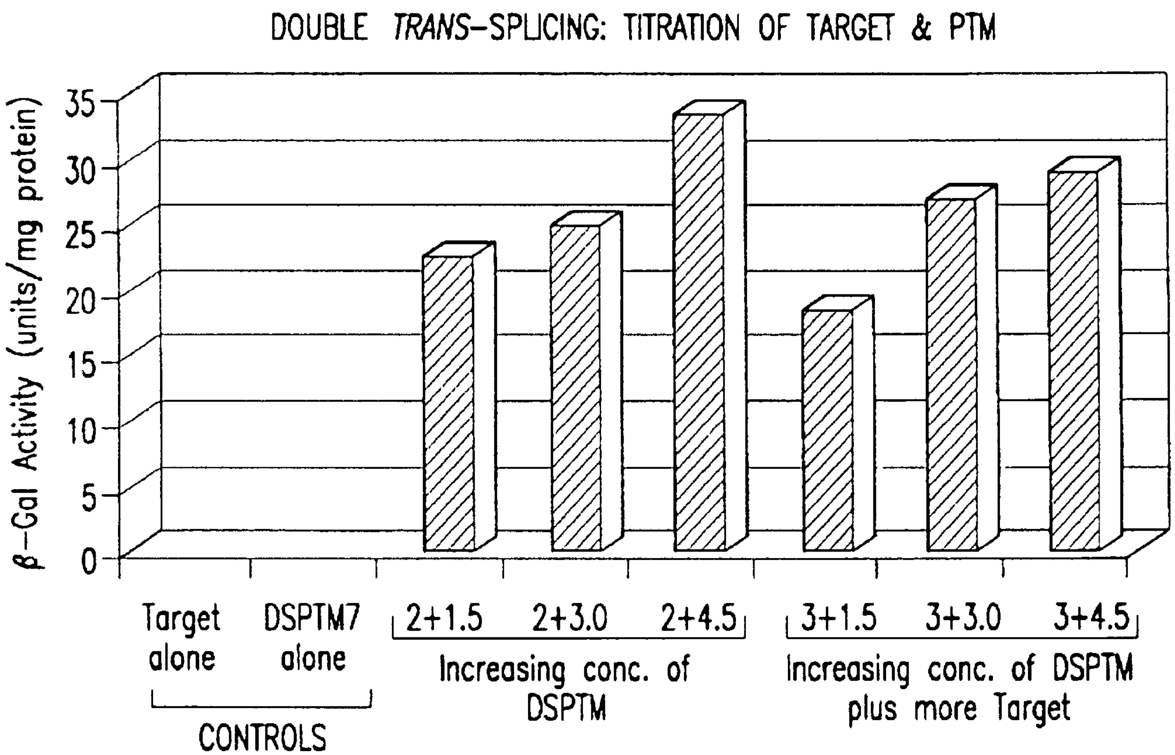

RESTORATION OF β-GAL ACTIVITY IS DUE TO DOUBLE RNA TRANS-SPLICING EVENTS
Beta-gal Activity (units/mg protein)
20
15
10
5
0
Target alone
DSPTM alone
Δ 3'ss alone
T1-6+ PTM29
T1-6+ PTM30
T1-6+29+ Δ 3'ss
T1-6+30+ Δ 3'ss
T1-6+DSPTM7
CONTROLS
Target (T1-6):
Lac 5' Ex
CF Ex 10
Lac 3' Ex
DSPTM7:
Mini Ex
DSPTM8 (Δ3'ss)
PTM30 (lacks 1st BD and 3'ss)
PTM29 (lacks 2nd BD and 5'ss)
Mutants

FIG.26



The current level of beta-gal activity due to double *trans*-splicing is ~ 1-1.5% of the best single splice model (3' exon replacement)

FIG.27

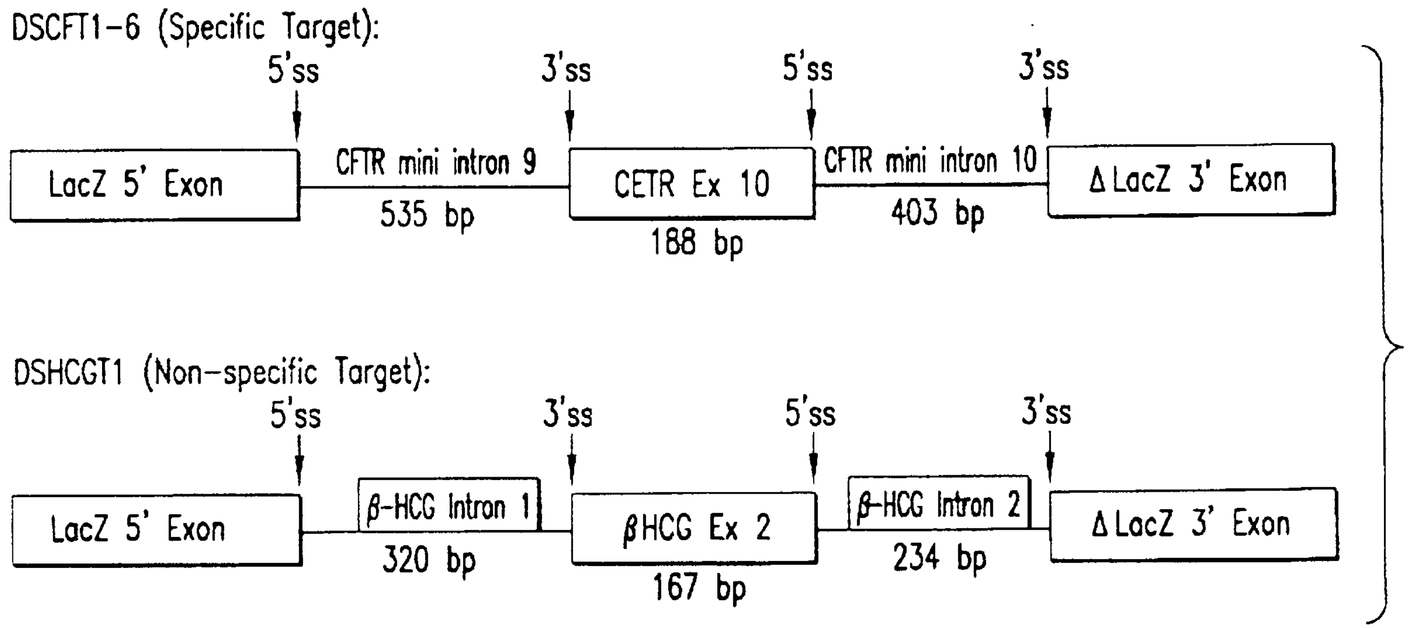

DSCFT1-6 (Specific Target):
5'ss
3'ss
5'ss
3'ss
LacZ 5' Exon
CFTR mini intron 9
535 bp
CETR Ex 10
188 bp
CFTR mini intron 10
403 bp
Δ LacZ 3' Exon
DSHCGT1 (Non-specific Target):
5'ss
3'ss
5'ss
3'ss
LacZ 5' Exon
β-HCG Intron 1
320 bp
βHCG Ex 2
167 bp
β-HCG Intron 2
234 bp
Δ LacZ 3' Exon

FIG.28

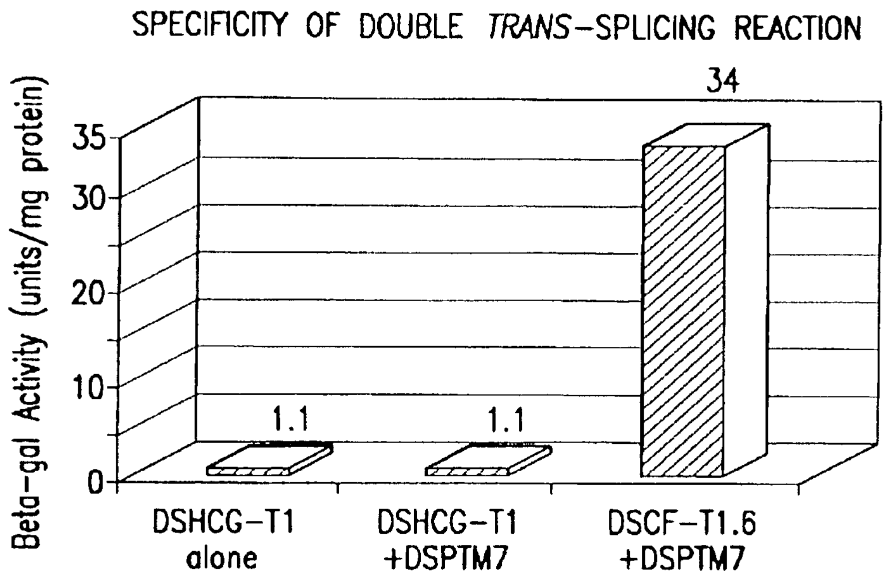

SPECIFICITY OF DOUBLE TRANS-SPLICING REACTION
Beta-gal Activity (units/mg protein)
35
30
20
10
0
1.1
1.1
34
DSHCG-T1 alone
DSHCG-T1 +DSPTM7
DSCF-T1.6 +DSPTM7

FIG.29

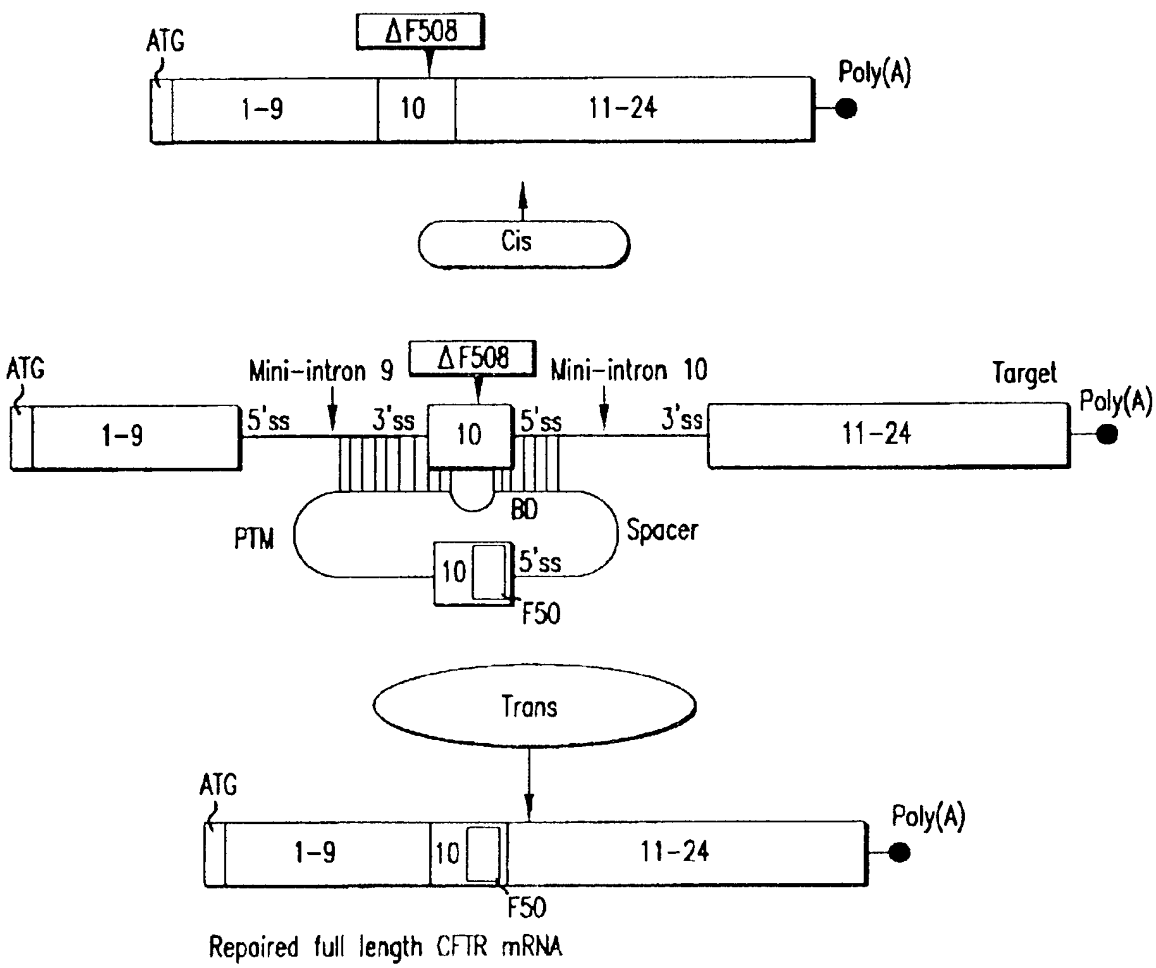
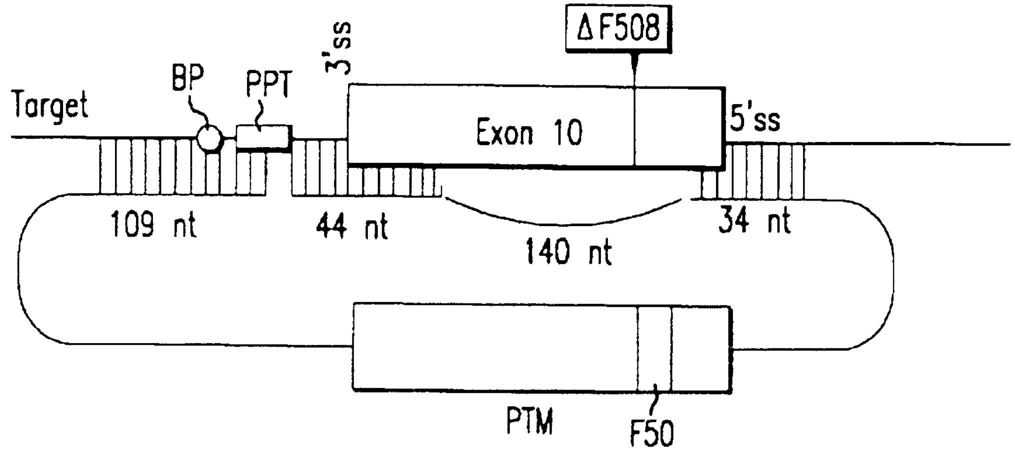

ATG
ΔF508
1-9
10
11-24
Poly(A)
Cis
ATG
Mini-intron 9
ΔF508
Mini-intron 10
Target
Poly(A)
1-9
5'ss
3'ss
10
5'ss
3'ss
11-24
BD
PTM
Spacer
10
5'ss
F50
Trans
ATG
Poly(A)
1-9
10
11-24
F50
Repaired full length CFTR mRNA

FIG.30

PTM with a long binding domain masking
two splice sites and part of exon 10
in a mini-gene target



ACGAGCTTGCTCATGATGATCATGGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAACATTCCG
GCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAACATAAT
CTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCTGTCAGTTGGAGGAG

MCU in exon 10 of PTM
88 OF 192 (46%) bases in PTM exon 10 are not complementary to its binding domain (bold and underlined).

FIG.31

Sequence of a double
Trans–spliced product

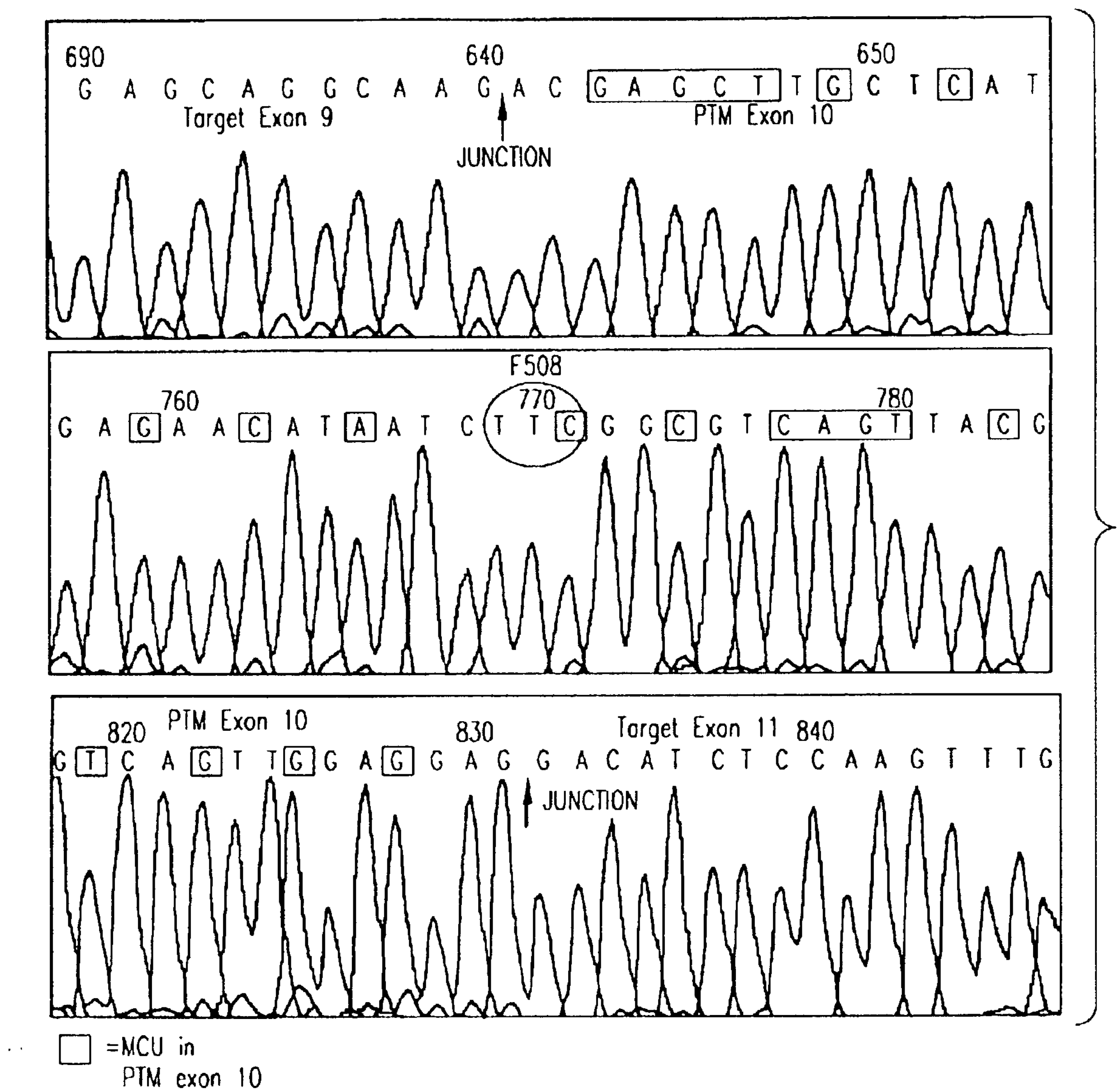


FIG.32

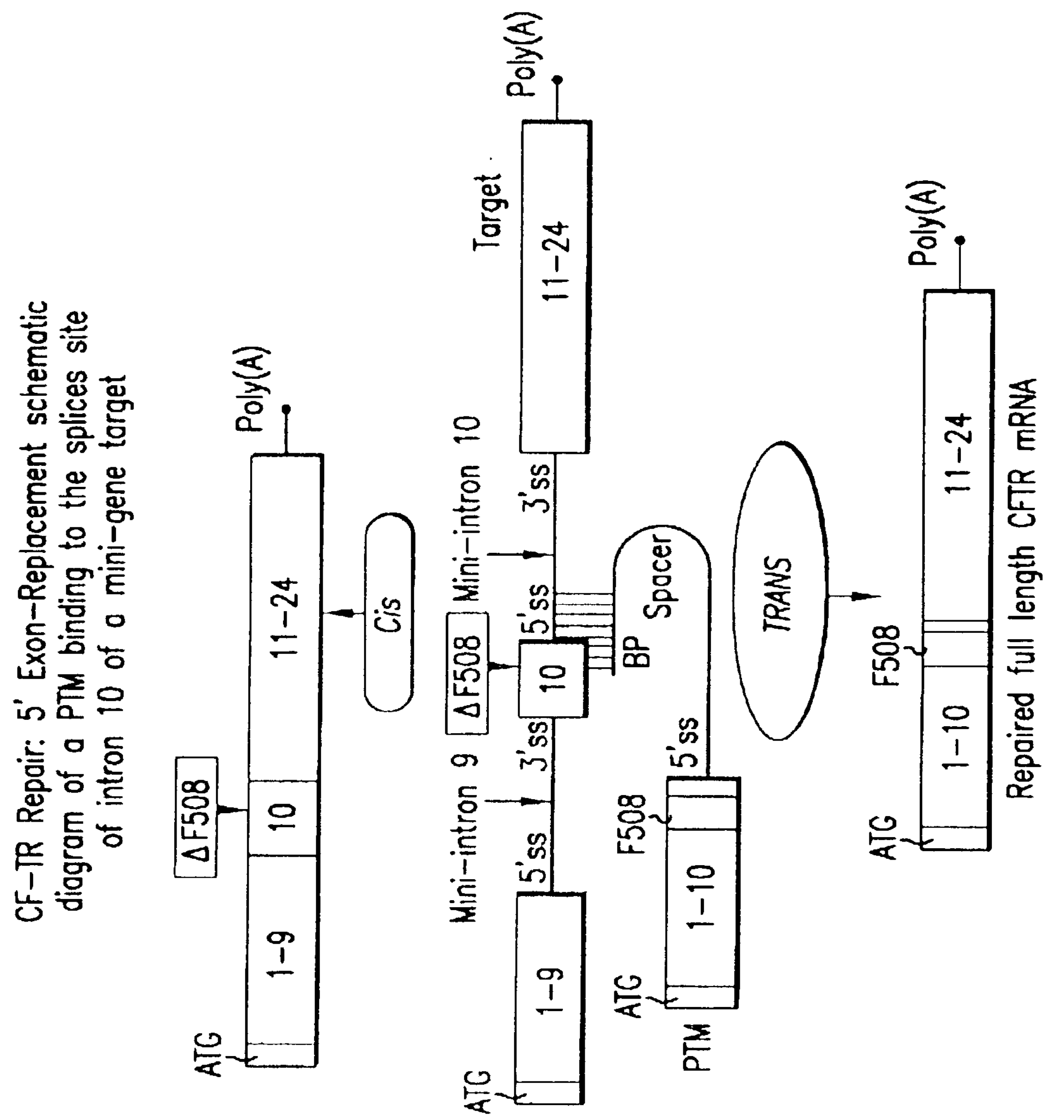

CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splices site of intron 10 of a mini-gene target
ATG
1-9
10
11-24
ΔF508
Poly(A)
Cis
ATG
1-9
Mini-intron 9
5'ss
3'ss
10
ΔF508
5'ss
Mini-intron 10
3'ss
11-24
Target
Poly(A)
BP
Spacer
PTM
ATG
1-10
F508
5'ss
TRANS
ATG
1-10
F508
11-24
Poly(A)
Repaired full length CFTR mRNA

FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target.

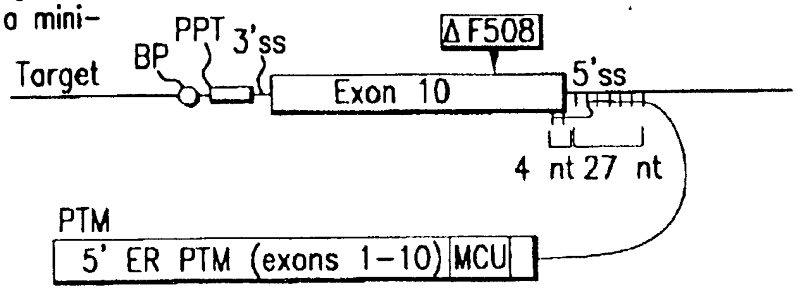


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

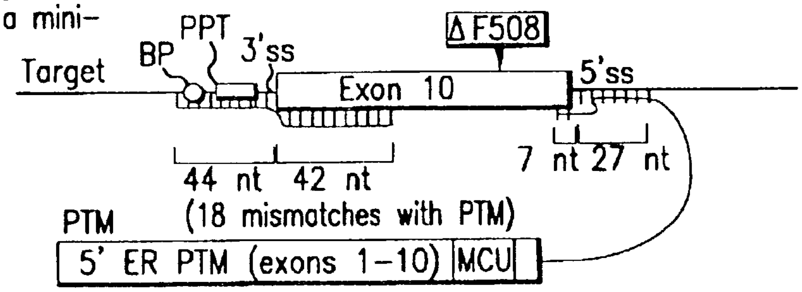


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

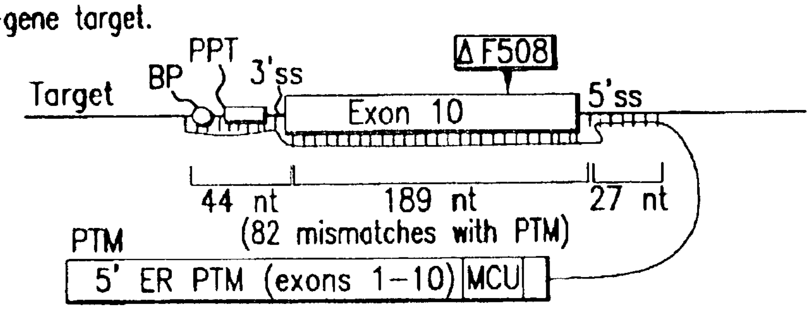
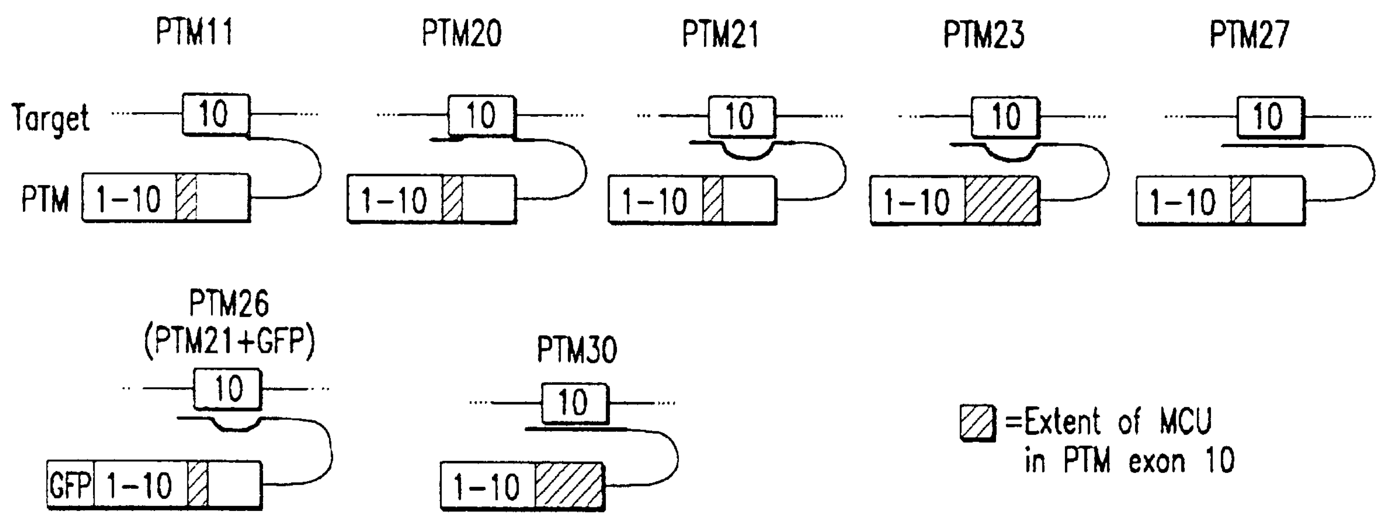


FIG.34C

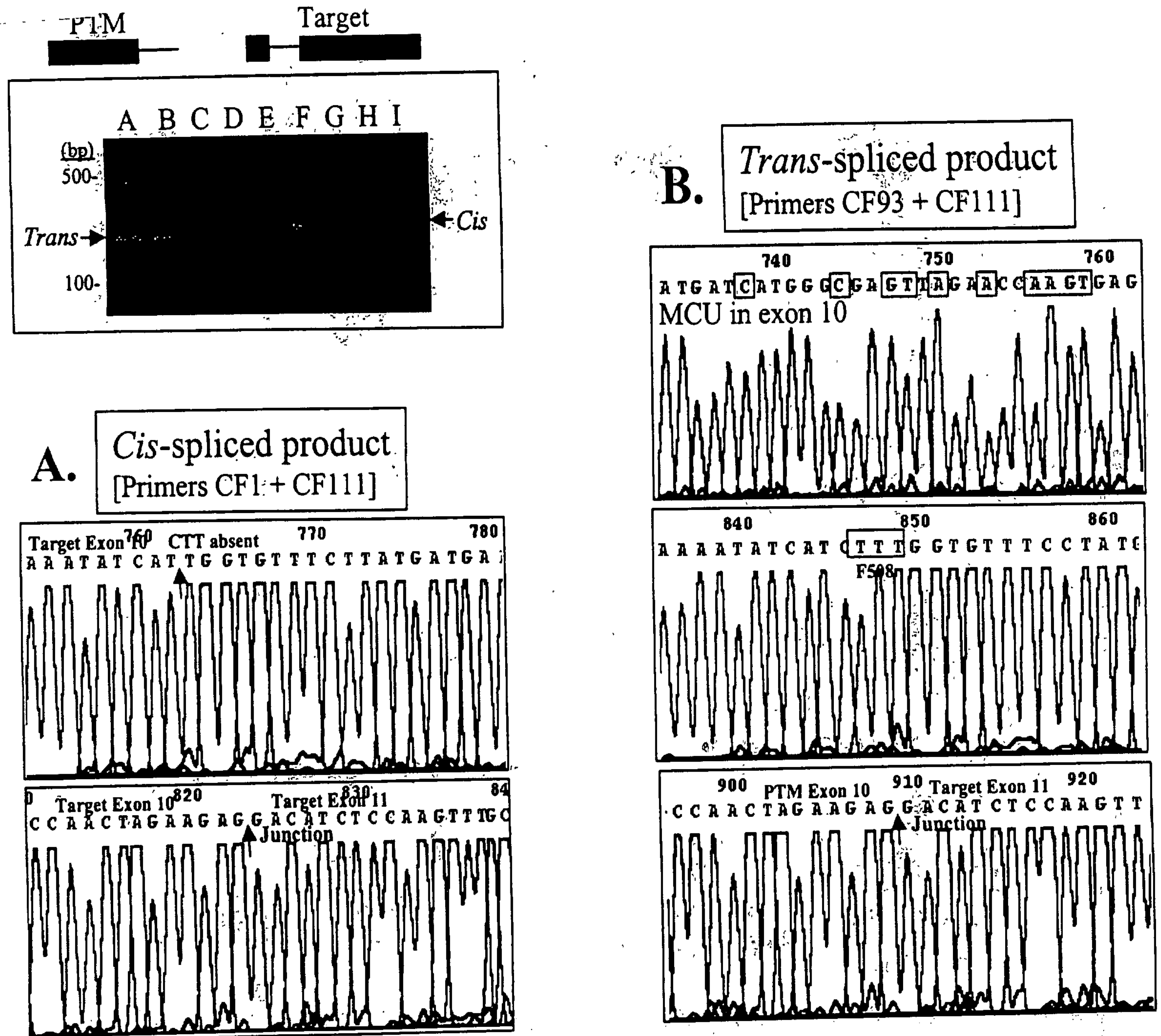


MCU in exon 10 of PTM
88 of 192 (46%) bases in PTM exon 10 are not complemetary to its binding domain.

ACGAGCTTGCTCATGATGATCATGGCCGAGTTAGAACCAAGTGAAGGCAAGATCAAACATTCCG
GCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAACATAAT
CTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCTGTCAGTTGGAGGAG

FIG.35

Figure 36


PTM
Target
A B C D E F G H I
(bp)
500-
100-
Trans
Cis
A.
Cis-spliced product
[Primers CF1 + CF111]
Target Exon 10
CTT absent
770
780
820
Target Exon 11
Junction
B.
Trans-spliced product
[Primers CF93 + CF111]
740
750
760
MCU in exon 10
840
850
860
F508
900
PTM Exon 10
910
Target Exon 11
920
Junction

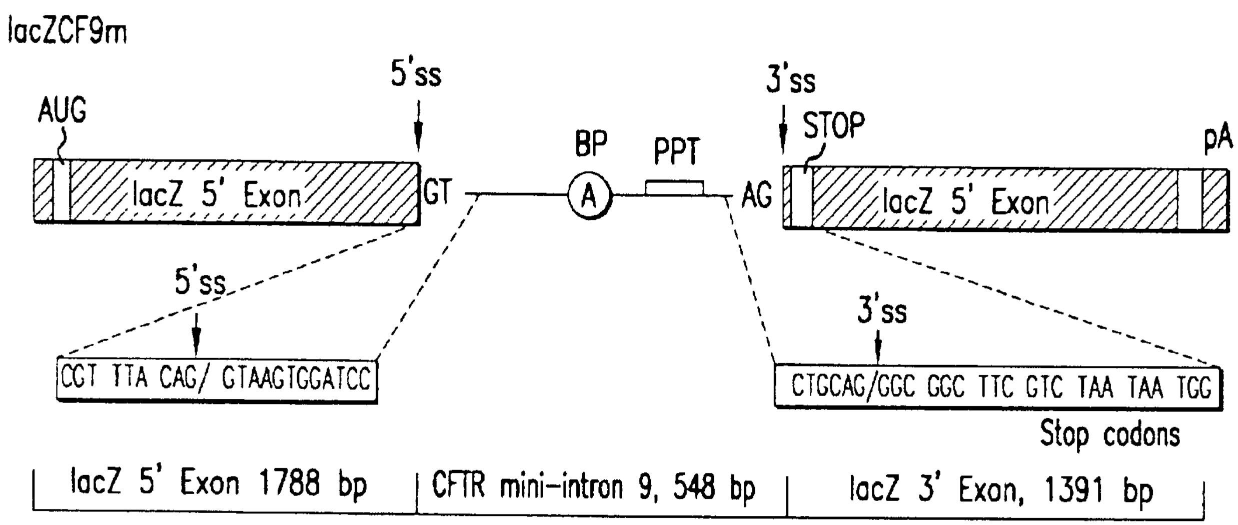

lacZCF9m
AUG
5'ss
lacZ 5' Exon
GT
BP
A
PPT
AG
3'ss
STOP
lacZ 5' Exon
pA
5'ss
CGT TTA CAG/ GTAAGTGGATCC
3'ss
CTGCAG/GGC GGC TTC GTC TAA TAA TGG
Stop codons
lacZ 5' Exon 1788 bp
CFTR mini-intron 9, 548 bp
lacZ 3' Exon, 1391 bp

FIG.37A

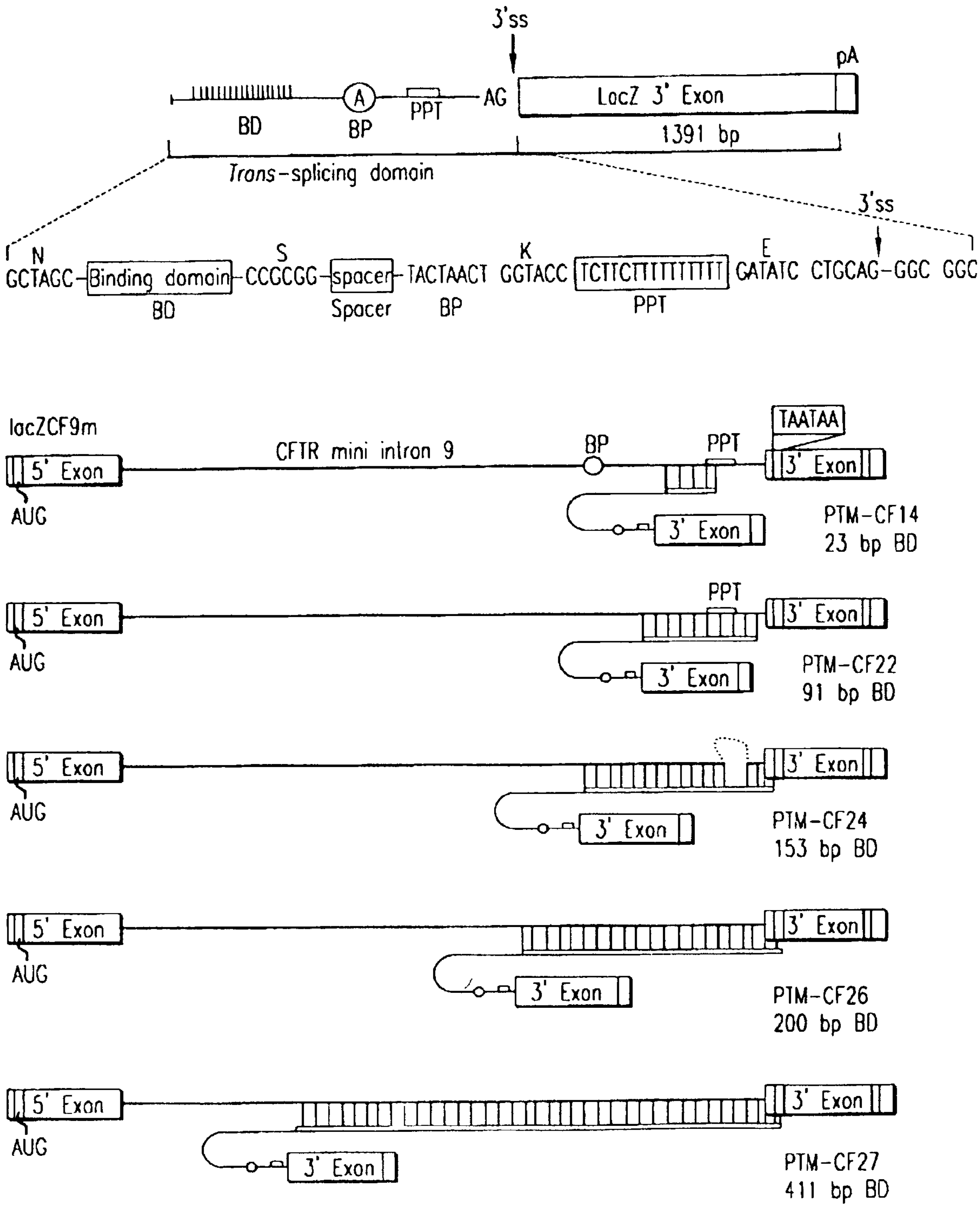

3'ss
pA
BD
BP
PPT
AG
LacZ 3' Exon
1391 bp
Trans-splicing domain
3'ss
N
S
K
E
GCTAGC
Binding domain
CCGCGG
spacer
TACTAACT
GGTACC
TCTTCTTTTTTTTTT
GATATC
CTGCAG-GGC GGC
BD
Spacer
BP
PPT
lacZCF9m
5' Exon
CFTR mini intron 9
BP
PPT
TAATAA
3' Exon
AUG
3' Exon
PTM-CF14
23 bp BD
PPT
PTM-CF22
91 bp BD
PTM-CF24
153 bp BD
PTM-CF26
200 bp BD
PTM-CF27
411 bp BD

FIG.37B

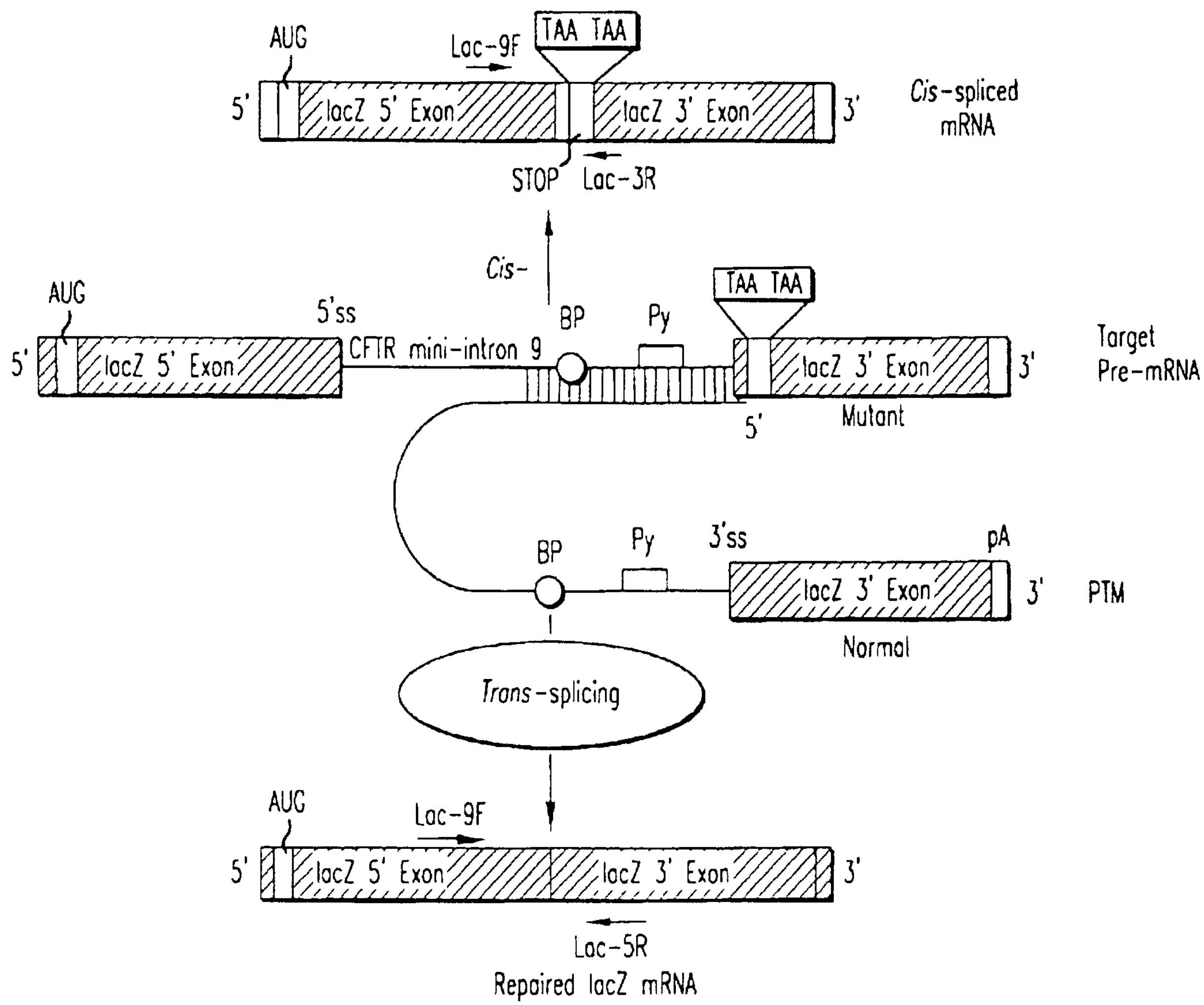

AUG
Lac-9F
TAA TAA
5'
lacZ 5' Exon
lacZ 3' Exon
3'
Cis-spliced
mRNA
STOP
Lac-3R
Cis-
AUG
5'ss
CFTR mini-intron 9
BP
Py
TAA TAA
5'
lacZ 5' Exon
lacZ 3' Exon
3'
Target
Pre-mRNA
Mutant
5'
BP
Py
3'ss
pA
lacZ 3' Exon
3'
PTM
Normal
Trans-splicing
AUG
Lac-9F
5'
lacZ 5' Exon
lacZ 3' Exon
3'
Lac-5R
Repaired lacZ mRNA

FIG.37C

A

Cis-splicing						Trans-splicing									
lacZCF9m + PTM-CF14												lacZCF9			
25 ng			50 ng			100 ng			200 ng			25 ng			Total RNA
20	25	30	20	25	30	20	25	30	20	25	30	20	25	30	# PCR cycles

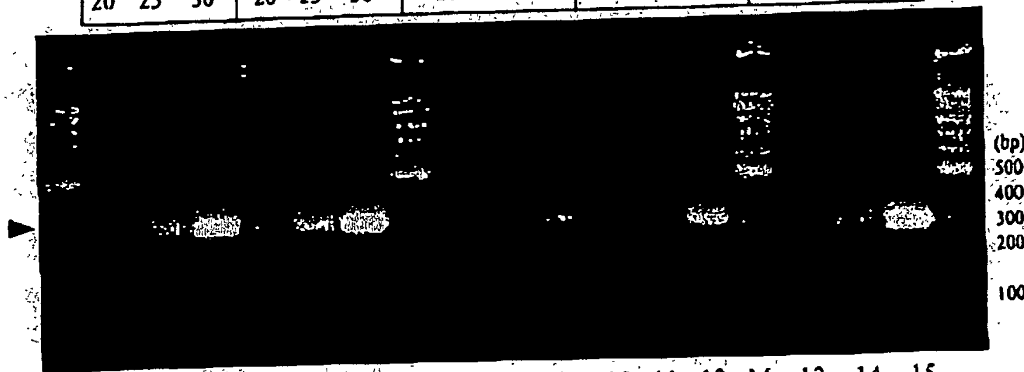
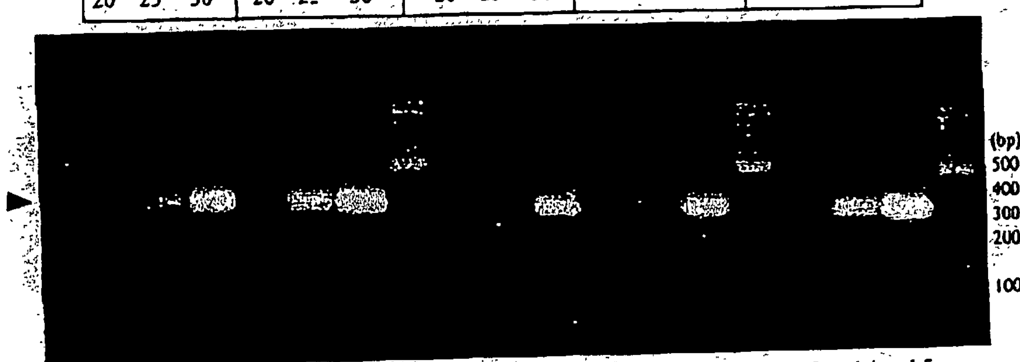


Figure 35 A

Cis-splicing						Trans-splicing									
lacZCF9m + PTM-CF24												lacZCF9			
25 ng			50 ng			50 ng			100 ng			50 ng			Total RNA
20	25	30	20	25	30	20	25	30	20	25	30	20	25	30	# PCR cycles



SCANNED. # 14

B

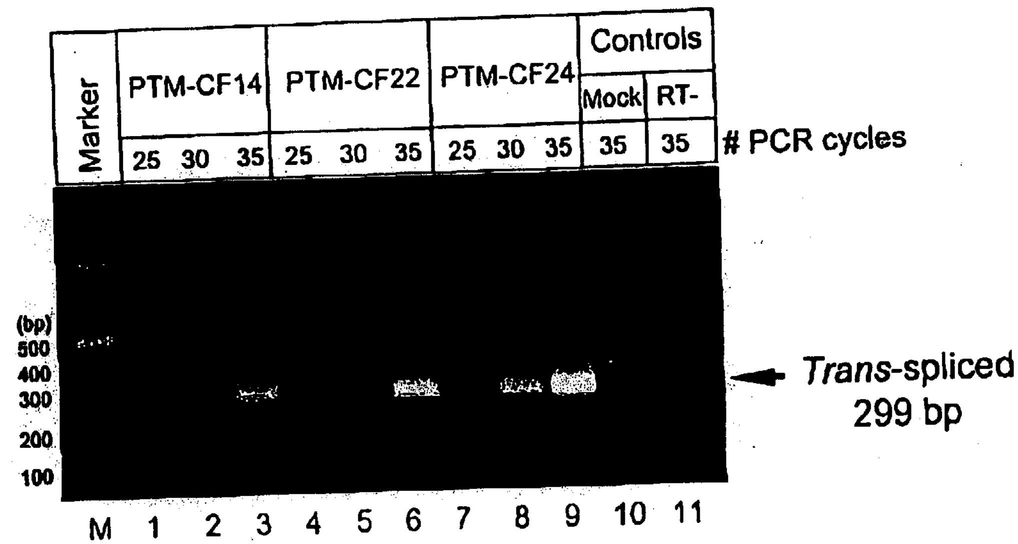


Figure 38B

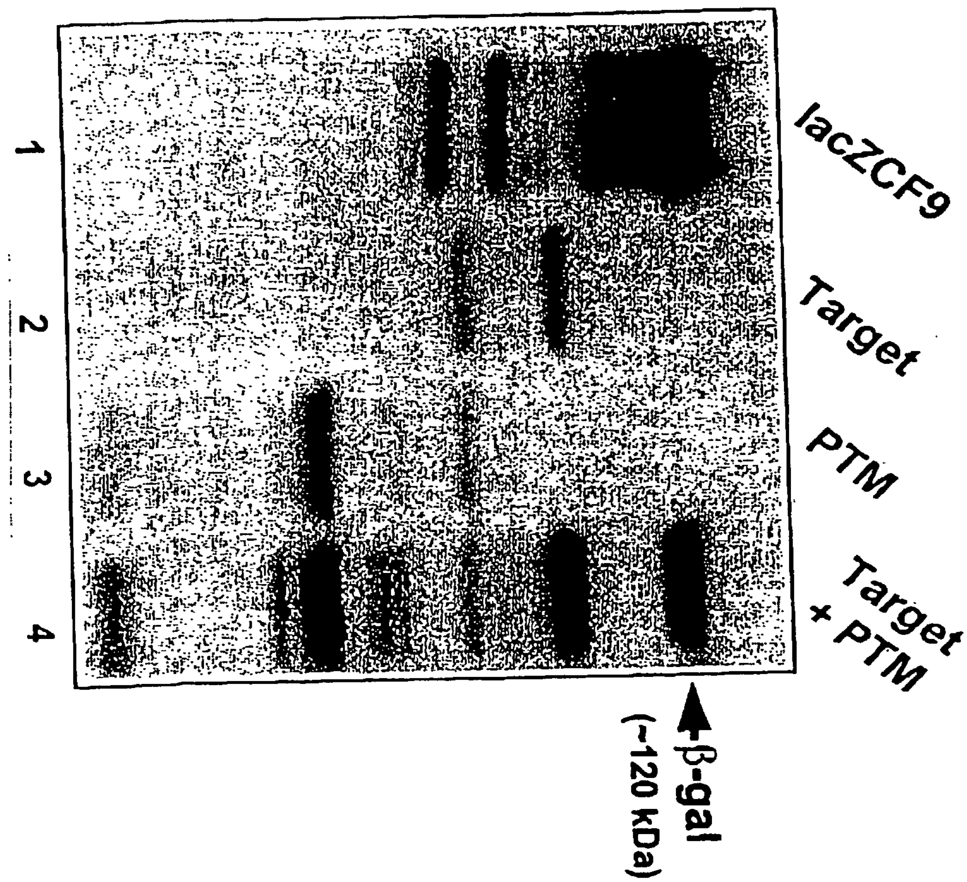


Figure 39

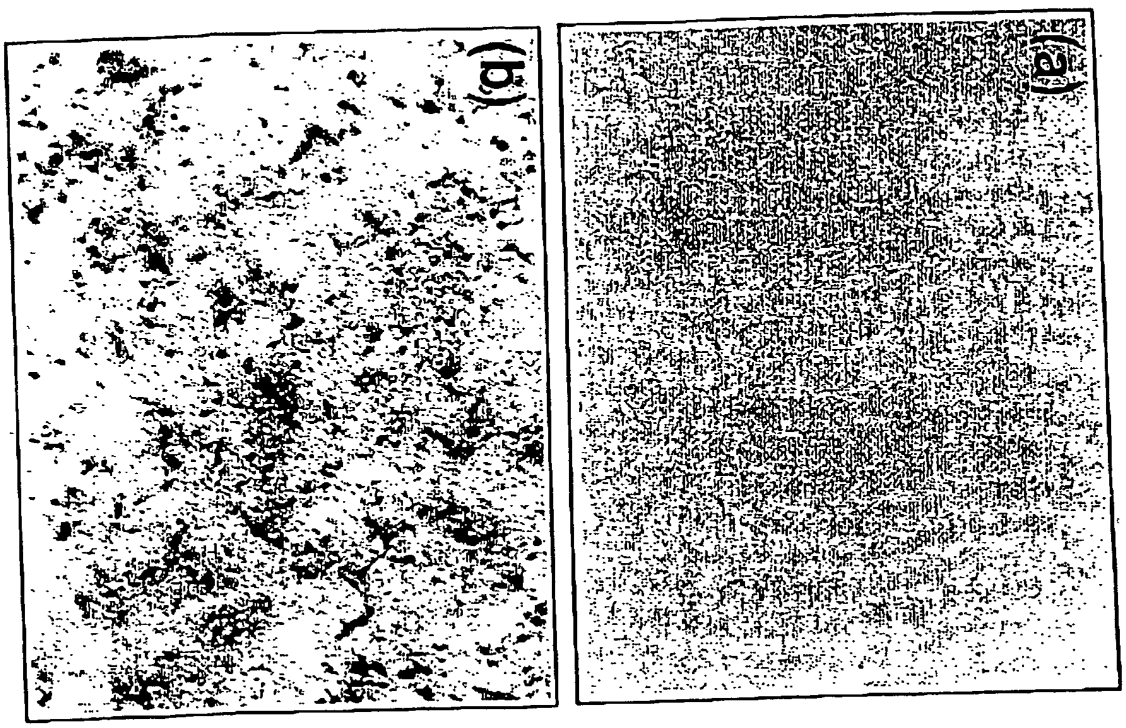


Figure 40A

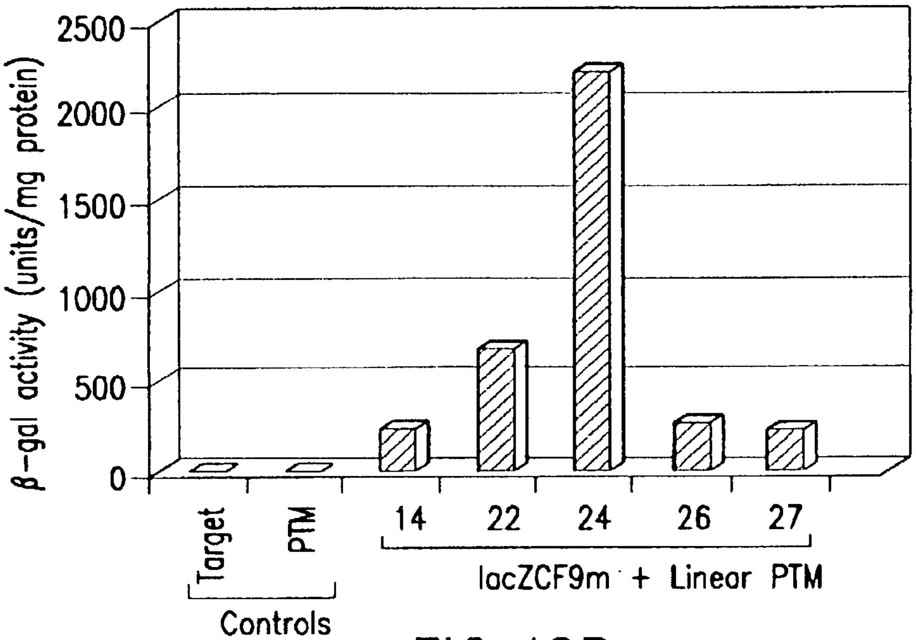


FIG.40B

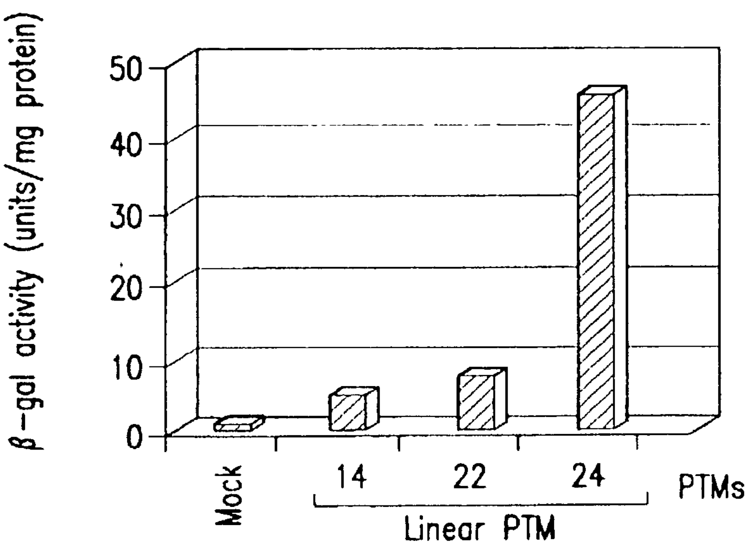


FIG.40C

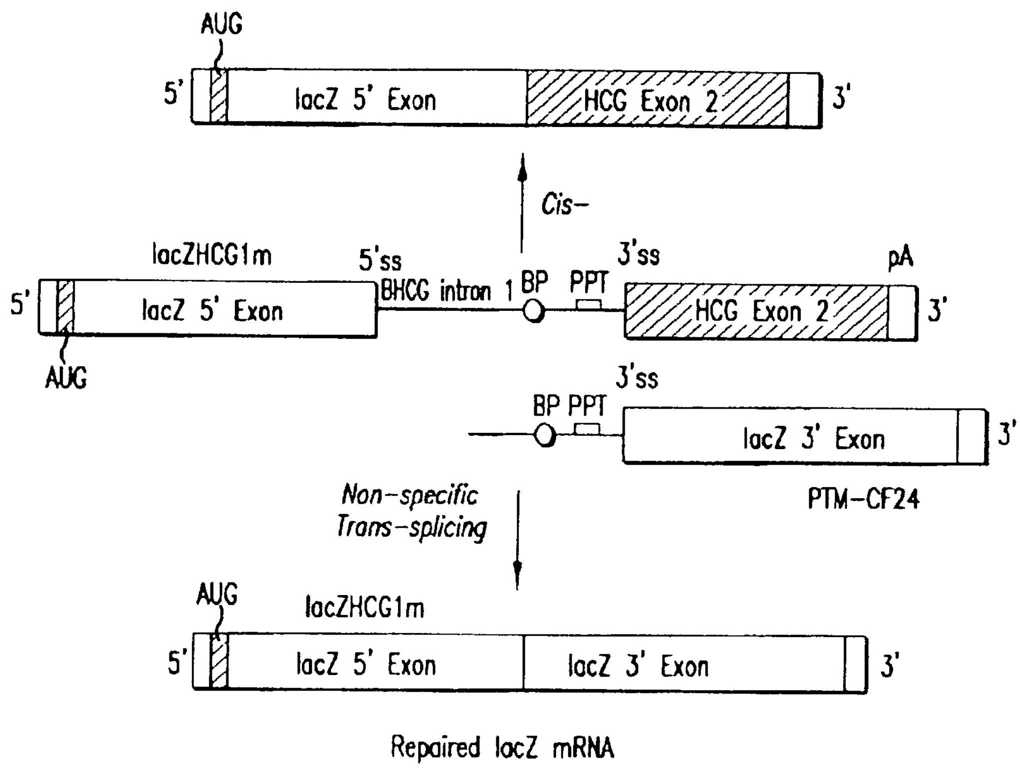

AUG
5'
lacZ 5' Exon
HCG Exon 2
3'
Cis-
lacZHCG1m
5'ss
3'ss
pA
5'
lacZ 5' Exon
BHCG intron 1
BP
PPT
HCG Exon 2
3'
AUG
3'ss
BP PPT
lacZ 3' Exon
3'
Non-specific
Trans-splicing
PTM-CF24
AUG
lacZHCG1m
5'
lacZ 5' Exon
lacZ 3' Exon
3'
Repaired lacZ mRNA

FIG.41A

...NED. # 14

B

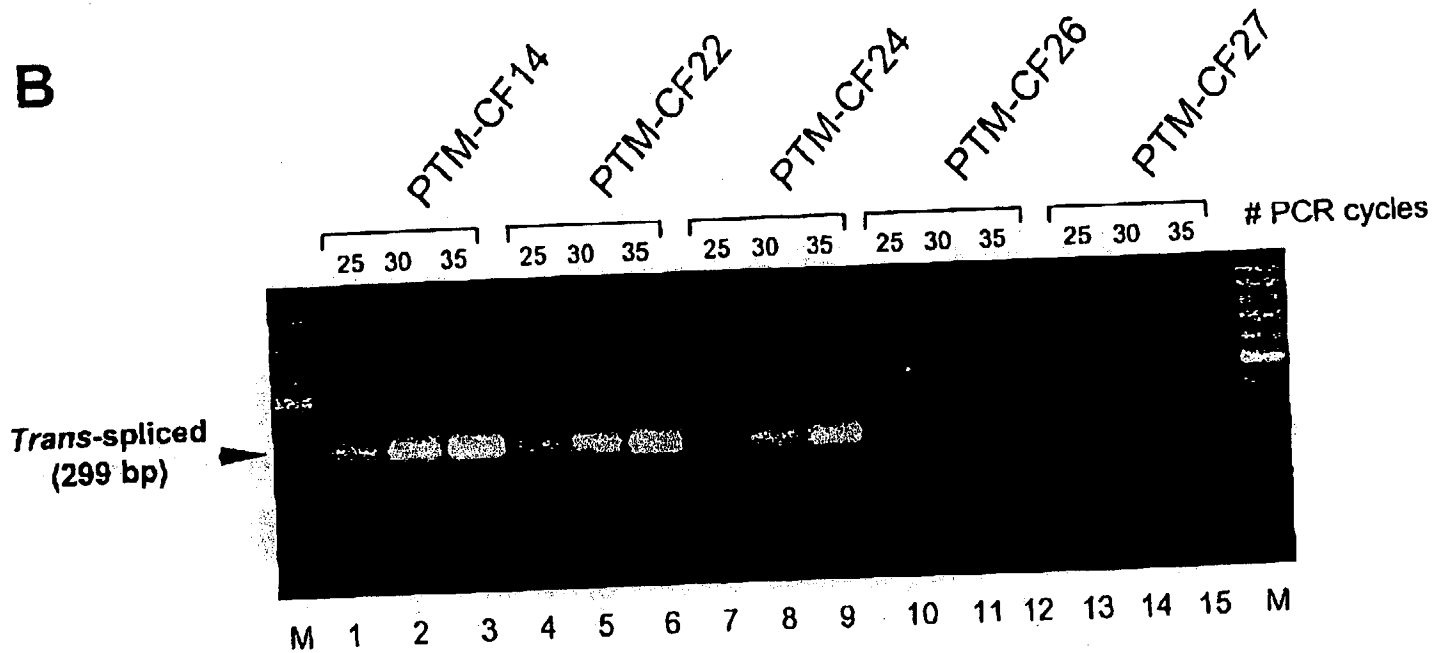


Figure. 4B

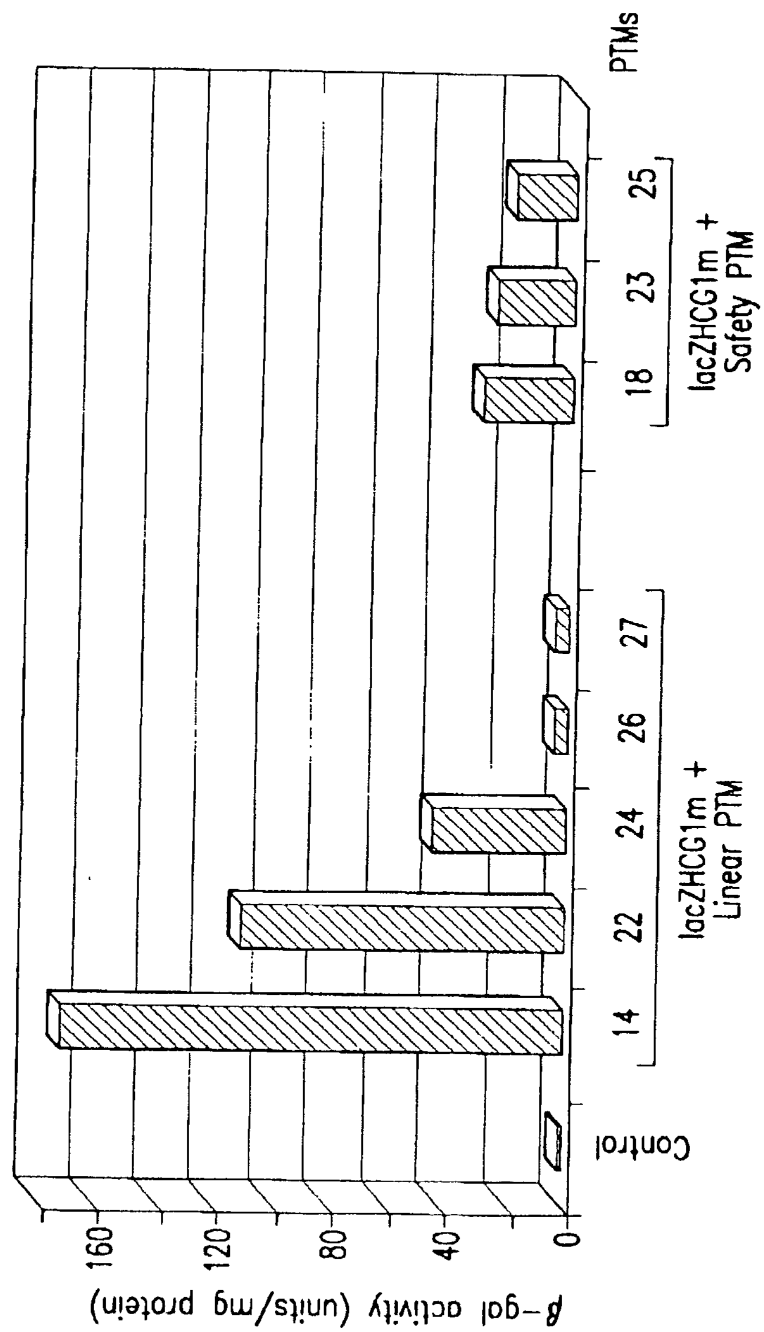

β-gal activity (units/mg protein)
0
40
80
120
160
Control
14
22
24
26
27
18
23
25
lacZHCG1m +
Linear PTM
lacZHCG1m +
Safety PTM
PTMs

FIG.41C

Exons
1-10

ATGCAGAGGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG
GATACAGACAGCGCCTGGAATTGTCAGACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT
GGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGATGTTTTTTCTGG
AGATTTATGTTCTATGGAATCTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA
TAGCTTCCTATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTAT
TGTGAGGACACTGCTCCTACACCCAGCCATTTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGT
TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACTTGTTAGTCTCCTTT
CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCACATTTCGTGTGGATCGCTCCTTTGCAAGTGGCACTCCT
CATGGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCCTGATAGTCCTTGCCCTTTTTCAG
GCTGGGCTAGGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG
AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGAAAAAATGATTGAAAACTTAAGACA
AACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTTCTTT
GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGGAAAATATTCACCACCATCTCATTCT
GCATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA
CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG
AATGTAACAGCCTTCTGGGAGGAGGGATTTGGGGAATTATTTGAGAAAGCAAAACAAAACAATAACAATAGAAAAACTT
CTAATGGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCTTGGTACTCCTGTCCTGAAAGATATTAATTTCAAGAT
AGAAAGAGGACAGTTGTTGGCGGTTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTGCTCATGATGATCATGGCCGAG
TTAGAACCAAGTGAAGGCAAGATCAAACATTCCGGCCCCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTA
CCATCAAGGAGAACATAATCTTCGGCGTCAGTTACGACGAGTACCGCTATCCCTCGGTGATTAAGGCCTGTCAGTTGGA
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACGCTAAGATCCACCGG
TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG
GAAACACCAATGATATTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGT
GCTTAATTTTACCCTCTGAATTCTCCATTTCTCCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATT
ATTAACTCATTATCAAATCACGCT

FIG.42

153 bp PTM24 Binding Domain:

Nhe I 153 bp BD underlined

GCTAGC-AATAATGACGAAGCCGCCCCTCACGCTCAGGATTCACTTGCCTCCAATTATCATCCTAAGCAGAAGTGTATA

TTCTTATTTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAAATACTTCCTGTTTCACCTACTCTGCTATGC

Sac II

AC-CCGCGG

FIG.43A

Trans-splicing domain
AATAATGACGAAGCCGCCCCTCACGCTCAGGATTCACTTGCCCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTA
TTTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAAATACTTCCTGTTTCACCTACTCTGCTATGCACCCGC
GGAACATTATTATAACGTTGCTCGAATACTAACTGGTACCTCTTCTTTTTTTTTTTGATATCCTGCAG
Exons10-24
ACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGGAAGAATTTCATTCT
GTTCTCAGTTTTCCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTTCCTATGATGAATATAGATA
CAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTTCTTGGAGAA
GGTGGAATCACACTGAGTGGAGGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTTGTATT
TATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAGAAATATTTGAAAGCTGTGTCTGTAAACTGATGGC
TAACAAAACTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCATGAAGGT
AGCAGCTATTTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAAACTCATGGGATGTGATT
CTTTCGACCAATTTAGTGCAGAAAGAAGAAATTCAATCCTAACTGAGACCTTACACCGTTTCTCATTAGAAGGAGATGC
TCCTGTCTCCTGGACAGAAACAAAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAAAGGAAGAATTCTATT
CTCAATCCAATCAACTCTATACGAAAATTTTCCATTGTGCAAAAGACTCCCTTACAAATGAATGGCATCGAAGAGGATT
CTGATGAGCCTTTAGAGAGAAGGCTGTCCTTAGTACCAGATTCTGAGCAGGGAGAGGCGATACTGCCTCGCATCAGCGT
GATCAGCACTGGCCCCACGCTTCAGGCACGAAGGAGGCAGTCTGTCCTGAACCTGATGACACACTCAGTTAACCAAGGT
CAGAACATTCACCGAAAGACAACAGCATCCACACGAAAAGTGTCACTGGCCCCTCAGGCAAACTTGACTGAACTGGATA
TATATTCAAGAAGGTTATCTCAAGAAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT
TTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCACAAGAGCTTA
ATTTTTGTGCTAATTTGGTGCTTAGTAATTTTTCTGGCAGAGGTGGCTGCTTCTTTGGTTGTGCTGTGGCTCCTTGGAA
ACACTCCTCTTCAAGACAAAGGGAATAGTACTCATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTC
GTATTATGTGTTTTACATTTACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATTCTTCAGAGGTCTACCACTGGTG
CATACTCTAATCACAGTGTCGAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCCTCA
ACACGTTGAAAGCAGGTGGGATTCTTAATAGATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGCCTCTTACCAT
ATTTGACTTCATCCAGTTGTTATTAATTGTGATTGGAGCTATAGCAGTTGTCGCAGTTTTACAACCCTACATCTTTGTT
GCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCCTCCAAACCTCACAGCAACTCAAACAACTGG
AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTTACAAGCTTAAAAGGACTATGGACACTTCGTGCCTTCGGACG
GCAGCCTTACTTTGAAACTCTGTTCCACAAAGCTCTGAATTTACATACTGCCAACTGGTTCTTGTACCTGTCAACACTG
CGCTGGTTCCAAATGAGAATAGAAATGATTTTTGTCATCTTCTTCATTGCTGTTACCTTCATTTCCATTTTAACAACAG
GAGAAGGAGAAGGAAGAGTTGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGGGCTGTAAACTC
CAGCATAGATGTGGATAGCTTGATGCGATCTGTGAGCCGAGTCTTTAAGTTCATTGACATGCCAACAGAAGGTAAACCT
ACCAAGTCAACCAAACCATACAAGAATGGCCAACTCTCGAAAGTTATGATTATTGAGAATTCACACGTGAAGAAAGATG
ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAAATGCCATATTAGA
GAACATTTCCTTCTCAATAAGTCCTGGCCAGAGGGTGGGCCTCTTGGGAAGAACTGGATCAGGGAAGAGTACTTTGTTA
TCAGCTTTTTTGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTGTCTTGGGATTCAATAACTTTGCAAC
AGTGGAGGAAAGCCTTTGGAGTGATACCACAGAAAGTATTTATTTTTTCTGGAACATTTAGAAAAAACTTGGATCCCTA
TGAACAGTGGAGTGATCAAGAAATATGGAAAGTTGCAGATGAGGTTGGGCTCAGATCTGTGATAGAACAGTTTCCTGGG
AAGCTTGACTTTGTCCTTGTGGATGGGGGCTGTGTCCTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG
TTCTCAGTAAGGCGAAGATCTTGCTGCTTGATGAACCCAGTGCTCATTTGGATCCAGTAACATACCAAATAATTAGAAG
AACTCTAAAACAAGCATTTGCTGATTGCACAGTAATTCTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA
TTTTTGGTCATAGAAGAGAACAAAGTGCGGCAGTACGATTCCATCCAGAAACTGCTGAACGAGAGGAGCCTCTTCCGGC
AAGCCATCAGCCCCTCCGACAGGGTGAAGCTCTTTCCCCACCGGAACTCAAGCAAGTGCAAGTCTAAGCCCCAGATTGC
Histidine tag Stop
TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATCATTAG

FIG.43B